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THE NUTRITION OF LETTUCE

BY R. M. WOODMAN, *Horticultural Research Station, Cambridge University*

In 1935, nutritional work on lettuce was started by the author. The investigations were divided into three sections: sand-culture experiments, soil-culture experiments, and field-fertilizer trials. The results of the sand-culture work have been published (Woodman, 1936, 1939 *a*, *b*, *c*, *d*, 1940 *a*, *b*); the present paper deals with the results obtained so far from soil cultures and field trials.

SOIL CULTURES OF LETTUCE

These experiments were made in 1935–6, before the sand-culture experiments mentioned above. The method used was the triangle one, which will be noted in the sequence to be far from ideal for the purpose.

Duplicate trials were made, with sixty-six cultures in each. The total soluble $P_2O_5 + N + K_2O$ was maintained at the fixed rate of 150 lb. p.a., while the three individual constituents were varied in all possible ways in steps of one-tenth. It is usual to neglect applications of binary mixtures and of single salts (i.e. the perimeter of the triangle, and the apices), and to experiment only with the thirty-six ternary mixtures. This seemed to be an unfair presentment of the problem, and the sixty-six possible combinations are here taken (cf. Schreiner & Skinner, 1918).

The pots used were new, unglazed plant pots, sixteen (large) to the cast. The soil was from the top 9 in. of the interference pathways of the outside plots described later; it was an old river gravel, sieved through a zinc grating with circular perforations 2 mm. diam., and then well mixed. In each pot was placed 12½ lb. of soil, this amount being 1/240,000th of 3,000,000 lb. (equivalent to the top 9 in. of an acre of sandy soil). Thus any amount of fertilizer applied should be 1/240,000th of that applied p.a. It was thought best to work from the weight of the soil per pot, rather than from the ratio (area of pot/acre), as the area of the tops of the pots varied to some extent, whereas the mass of soil could be maintained constant, and the depth was then approximately 9 in. in each pot.

The soil in the pots was thoroughly leached four times a day with rain water for about a month, in an effort to make it 'poor'. After a few days free from leaching, the lettuce (May King) was sown on 28 Oct. 1935. Germination took place by 4 Nov. 1935, and the seedlings were singled to one per pot on 28 Nov. On the day after the sowing, the fertilizer mixtures were applied to each pot mixed with 25 g. of sand containing 99·8% SiO_2 (Woodman, 1936). Table 1, cultures A1–A66, shows the amounts in lb. of commercial ammonium sulphate (20·6% N), superphosphate (13·75% sol. P_2O_5), and potassium sulphate (50% K_2O) p.a. in the applied fertilizer mixtures, equivalent to a constant total of 150 lb. of sol. $P_2O_5 + N + K_2O$ p.a. The actual amounts per pot are omitted, but are, as has already been stated, 1/240,000th part of the quoted amounts p.a. Rain water was then applied to the cultures at intervals throughout the experiments in amounts such as to keep the soil moist, but to avoid leaching. Pots A1–A66, representing the first triangle, were randomized among themselves in the greenhouse; B1–B66 were exact duplicates of these, and were also randomized among themselves.

In addition to these 132 cultures, as the single salt applications representing the apices of the triangle were very heavy, it was resolved to add extra pots in which the salt varied in steps of one-fifth. In this manner were obtained cultures C1–C12 of Table 1, C1–C4 for nitrogen, C5–C8 for phosphate, and C9–C12 for potash. These pots acted as a kind of control, though they could not really be included with the triangle results. Pots C13–C66 received only the 25 g. of sand each. These also could not be included in the triangle, as one of the drawbacks of the triangle method is that each treatment is constant in weight of sol. $P_2O_5 + N + K_2O$, so that there is no room for the NIL (or 000) treatment. C13–C66, however, constituted a general datum line, and these cultures and C1–C12 were distributed at random throughout the cultures of the two triangles.

Because of the incidence of *Botrytis* on one or two cultures, the lettuces were harvested on 17 Mar. 1936. They were cut off at soil level and weighed. Only 14% of the controls (000 treatment) showed signs of hearting; but 44% of those receiving some fertilizer did, of which eight lettuces were fully hearted. At the outset, therefore, it seemed obvious that applications of fertilizer tended to earlier maturity.

TABLE 1. *Lb. p.a. of fertilizer salts equivalent to the amounts used in the pots, and, for the cultures* A1–A66 *and* B1–B66, *to a constant total of sol.* $P_2O_5 + N + K_2O = 150$ *lb. p.a.* (N = *ammonium sulphate*, P = *superphosphate, and* K = *potassium sulphate*). *Also treatment means* (T.M.) *for the lettuces in g. Variety, May King*

Culture A and B	N	P	K	T.M.	Culture A and B	N	P	K	T.M.
1	728	0	0	59·25	41	146	436	120	49·25
2	655	0	30	62·75	42	146	546	90	54·40
3	655	109	0	53·95	43	146	655	60	60·90
4	583	0	60	58·60	44	146	764	30	45·25
5	583	109	30	63·70	45	146	873	0	48·75
6	583	218	0	57·75	46	73	0	270	35·95
7	510	0	90	57·55	47	73	109	240	49·55
8	510	109	60	66·90	48	73	218	210	54·65
9	510	218	30	78·15	49	73	327	180	33·90
10	510	327	0	87·70	50	73	436	150	54·50
11	437	0	120	59·80	51	73	546	120	47·95
12	437	109	90	55·85	52	73	655	90	42·10
13	437	218	60	57·30	53	73	764	60	43·35
14	437	327	30	41·50	54	73	873	30	50·55
15	437	436	0	48·15	55	73	982	0	37·90
16	364	0	150	52·70	56	0	0	300	47·40
17	364	109	120	49·50	57	0	109	270	42·55
18	364	218	90	43·65	58	0	218	240	42·10
19	364	327	60	47·45	59	0	327	210	50·75
20	364	436	30	54·95	60	0	436	180	55·65
21	364	546	0	45·25	61	0	546	150	69·45
22	291	0	180	43·05	62	0	655	120	43·55
23	291	109	150	63·60	63	0	764	90	51·90
24	291	218	120	50·80	64	0	873	60	39·00
25	291	327	90	49·90	65	0	982	30	40·40
26	291	436	60	57·65	66	0	1091	0	38·60
27	291	546	30	66·95	C 1	583	0	0	47·4
28	291	655	0	34·45	C 2	437	0	0	51·5
29	218	0	210	42·60	C 3	291	0	0	41·7
30	218	109	180	43·25	C 4	146	0	0	44·8
31	218	218	150	70·85	C 5	0	873	0	34·0
32	218	327	120	46·05	C 6	0	655	0	56·0
33	218	436	90	46·50	C 7	0	436	0	39·6
34	218	546	60	40·45	C 8	0	218	0	52·1
35	218	655	30	62·45	C 9	0	0	240	58·9
36	218	764	0	64·35	C 10	0	0	180	45·9
37	146	0	240	46·65	C 11	0	0	120	35·7
38	146	109	210	44·60	C 12	0	0	60	50·8
39	146	218	180	40·90	C 13–C 66	0	0	0	41·79
40	146	327	150	45·40					

Plotting the results for triangle A or B on triangular graph paper (C.S. and S. no. 315½) demonstrated that there was no readily perceived correlation or regularity. It was therefore resolved to analyse the data of the triangles as if they were duplicates of a randomized block of sixty-six treatments. The treatment means are included in Table 1 for this purpose,

and it was found that z just showed significance at the 5% point, so that further analysis was possible. The S.E. of the treatment means was 8·117.

Further analysis of the results was made by isolating the effects of grouped treatments. The triangle is not reproduced, but a brief explanation of its arrangement may be given:

Treatment 1 (100% N, equivalent to 150 lb. p.a. of N, with no phosphate or potash) occupied point 1 at the apex at the summit of the equilateral triangle. The treatments (or points) then descended the triangle in the regular order 1–66, reading from left to right horizontally at each 10% variation of N. Thus 56 represented 100% K_2O at the left-hand lower apex, while 66 represented 100% sol. P_2O_5 at the right-hand lower one. The base of the triangle, points 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, and 66, denoted binary mixtures of sol. $P_2O_5 + K_2O$ with a constant total equivalent to 150 lb. p.a. (PK treatments); the side, 1, 3, 6, 10, 15, 21, 28, 36, 45, 55, and 66, denoted binary mixtures of N + sol. P_2O_5 equivalent to 150 lb. p.a. (NP treatments); and the side, 1, 2, 4, 7, 11, 16, 22, 29, 37, 46, and 56, denoted binary mixtures of $N + K_2O$ equivalent to 150 lb. p.a. (NK treatments). The thirty-six interior treatments or points represented ternary mixtures of sol. $P_2O_5 + N + K_2O$ equivalent always to 150 lb. p.a.

The following isolations were made on the treatment totals, which are twice the treatment means of Table 1; the general mean of these totals was 102·82 g.; the mean treatment totals for the grouped treatments are given in parentheses after the names of the groups at the beginning of each section:

NP treatments (104·75 *g.*) *v. all other treatments:* ns; within NP: ss; within others: ns. Where ns means no significance by the z test, and s, ss, and sss denote significance at the 5, 1, and 0·1% level, respectively.

The treatments within NP differed significantly at the 1% point. It was obvious, for instance, that treatments 10 and 36, with 30% sol. P_2O_5 and 70% N, and 70% sol. P_2O_5 and 30% N, differed significantly from some of the other NP treatments according to Table 1 (S.E. of treatment means, 8·117). Thus the proportions of N and sol. P_2O_5 have an effect on the yield of lettuce in the absence of potash.

NK treatments (102·96 *g.*) *v. all other treatments:* ns; within NK: ns; within others: s.

PK treatments (94·79 *g.*) *v. all other treatments:* ns; within PK: sss; within others: s.

The significance within the binary treatments for PK may be perceived from Table 1, S.E. 8·117, where treatment 61 with 50% each of sol. P_2O_5 and K_2O is significantly greater than treatments 64, 65, and 66, near the sol. P_2O_5-apex, and 57 and 58. In the absence of nitrogen, the proportions of P_2O_5 and K_2O have an effect, a response being got by 50% of each over ratios more in favour of sol. P_2O_5 or K_2O. The significance within others shows that there are significant differences between other treatments than the PK ones in the triangle, as indeed was shown by the significance 'within NP', got before.

The whole of the triangle was now divided into four subtriangles, three at the apices, and one central one, to try to isolate groups of treatments significantly different from the others.

Central small triangle (*treatments* 24, 25, 26, 32, 33, *and* 41, *Table* 1, *mean treatment total,* 100·05 *g.*) *v. all others:* ns; within these treatments: ns; within others: s.

Treatments in the N-apex triangle (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, *and* 21, *Table* 1, *mean treatment total,* 114·51 *g.*) *v. all others:* sss; within these treatments: s; within others: ns.

Treatments in the sol. P_2O_5*-apex triangle* (21, 27, 28, 34, 35, 36, 42, 43, 44, 45, 51, 52, 53, 54, 55, 61, 62, 63, 64, 65, *and* 66, *Table* 1, *mean treatment total,* 97·90 *g.*) *v. all others:* ns; within these treatments: ns; within others: s.

Treatments in the K_2O*-apex triangle* (16, 22, 23, 29, 30, 31, 37, 38, 39, 40, 46, 47, 48, 49, 50, 56, 57, 58, 59, 60, *and* 61, *Table* 1, *mean treatment total,* 98·10 *g.*) *v. all others:* ns; within these treatments: ns; within others: s.

The last four isolations demonstrate that the treatments in the triangle at the N-apex were significantly greater than all others, as is borne out by the fact that 'within others' here there was no significant difference. The general mean of the whole of the sixty-six treatment totals was 102·82 g.; the mean for the N-apex triangle, 1–21, was 114·51 g.: while that for the other forty-five treatments of the other parts of the original triangle, 22–66, was 97·37 g. No other part of the original triangle, that is, no other *logical* grouping of any of the sixty-six treatments, was found to give a significant difference above the other treatments.

Thus mixtures of fertilizers 1–21 were superior to the group 22–66. That is, the group of fertilizers containing 75–150 lb. N (or 364–728 lb. $(NH_4)_2SO_4$), 0–75 lb. sol. P_2O_5 (or 0–546 lb. of 13·75% sol. superphosphate), and 0–75 lb. K_2O (or 0–150 lb. K_2SO_4), gave a better response than the group of fertilizers containing 0–75 lb. N (or 0–364 lb. $(NH_4)_2SO_4$), 0–150 lb. sol. P_2O_5 (or 0–1091 lb. superphosphate), and 0–150 lb. K_2O (or 0–300 lb. K_2SO_4), when the total sol. $P_2O_5 + N + K_2O$ was maintained equivalent to 150 lb. p.a., and the sol. P_2O_5, N, and K_2O varied in 10% steps. Nitrogen, therefore, seems most necessary for lettuce on this soil.

There was now the problem of introducing the extra treatments randomized throughout the triangle experiments, and these were compared among themselves, and with cultures in the triangles, by the '*t*' test. C13–C66, fifty-four pots, Table 1, was the control, NIL, or 000 treatment; C1–C4 plus the two cultures for treatment 1 at the N-apex of the two triangles constituted the N treatments, with ammonium sulphate varying between 146 and 728 lb. p.a.; C5–C8 plus the two cultures 66 at the sol. P_2O_5-apex were the P treatments, with superphosphate varying from 218 to 1091 lb. p.a.; and C9–C12 with the two cultures 56 at the K_2O-apex made up the K treatments, with K_2SO_4 varying from 60 to 300 lb. p.a. The NP, NK, and PK binary mixtures are the sides of the triangles opposite the K_2O, sol. P_2O_5, and N, apices of the triangles, respectively, and have been previously discussed. The results of the analyses are set out in Table 2.

TABLE 2. *Mean yield per culture in g.*

	000	N	P	K	NP	NK	PK	A1–A66 and B1–B66
Mean yield	41·79	50·65	43·15	46·93	52·37	51·48	47·39	51·41
000	—	s	ns	ns	ss	ss	s	ss

With the soil used, ammonium sulphate alone (N) in varying proportions yielded a larger lettuce than no fertilizer (000), but there was no response to either superphosphate (P) or potassium sulphate (K) when used alone. Here again is evidence that the soil needs nitrogen for this crop. All the possible binary combinations of fertilizers, NP, NK, and PK, yielded significantly larger lettuces than the NIL (000) cultures. The results of all possible combinations of N, P, and K, according to the triangle method (A1–A66 and B1–B66), also gave a highly significant response over the control.

The following comparisons were all ns: N *v.* P, or K, or NP, or NK, or PK, or (A1–A66 and B1–B66); P *v.* K, or NP, or NK, or PK, or (A1–A66 and B1–B66); K *v.* NP, or NK,

or PK, or (A 1–A 66 and B 1–B 66); NP *v.* NK, or PK, or (A 1–A 66 and B 1–B 66); NK *v.* PK, or (A 1–A 66 and B 1–B 66); and PK *v.* (A 1–A 66 and B 1–B 66).

Field trials with lettuce

These were conducted on an old river gravel at the Horticultural Research Station, Cambridge. They comprised part of a comprehensive series of demonstration trials designed to show the effects of dung, N, P, and K, on the rotation of crops, sprouts, peas, summer lettuce (Feltham King) followed in the autumn by spring cabbage, which was in turn followed by early carrots sown late and taken off in the same year, so that there was a rotation of the five crops every 4 years. The rotation was introduced into the scheme to avoid any possible 'soil sickness' which might occur with one crop only in a permanent trial, and to approximate more closely to practice. As the plots were partly for demonstration purposes, the rotation was entered at three points, so that one crop was always 'on'. The limitations imposed by this and the lack of a sufficiency of land, coupled with the fact that permanent trials might conceivably be expected to lead eventually to obvious differences, resulted in the non-statistical, cyclical lay-out, NPK, NP, NK, PK, and NIL, dunged and undunged, so that there were $3 \times 5 = 15$ dunged, and 15 undunged, plots.

The fertilizers applied to the plots were a standard set drawn up for the different crops. For lettuce these were at the rate p.a. of 2 cwt. of ammonium sulphate, 5 cwt. of superphosphate, and 2 cwt. of potassium sulphate, the mixture being raked in the day before sowing. To the nitrogen plots, NPK, NP, and NK, a side-dressing of 2 cwt. p.a. of sodium nitrate was given, avoiding contact with the leaves, before turning in started.

As the rotation had been entered at three points, the three NPK plots, and similarly the three NP, NK, and PK, plots, were not exact triplicates, though each in a rotation had supported all the five crops in the rotation, and were triplicates in the sense that the three had received exactly the same total treatment during the whole of the rotation, the only variation being the year in which each of the three received the treatment for any particular crop. The three harvests for lettuce from the three NPK, etc., plots in every 4 years are therefore treated as replications. The lettuces were not transplanted, but were singled to 1 ft. each way. There were 324 lettuces per plot of 1/100th acre. Only marketable heads were cut, in eight standard cuts designed to test earliness. The total yields are given in Table 3 for the undunged plots.

Table 3. *Total yield of marketable lettuce (Feltham King) in lb. per 1/100th acre undunged plot*

Season	NPK	NP	NK	PK	NIL
1935	184	243	219	198	133
1936	270	205	250	236	232
1937	202	204	204	176	159
1938	—	—	—	—	—
1939	368·8	341·2	348·1	267·6	237·8
1940	221·7	105·1	178·4	209·0	26·5
1941	262·9	45·4	182·3	195·7	10·0
1942	—	—	—	—	—
Mean yields	251·57	190·62	230·30	213·72	133·05

The yields were compared by the table of '*t*'. For the first four harvests there was no significance, and it is obvious from the table that serious changes begin to occur only with the fifth harvest. The results of the tests of significance for the six harvests are given in Table 4.

In the absence of dung, and on the given soil, only the full artificials (NPK) have so far given a significant response over the NIL plots, though it is apparent from Table 3 that serious differences in yield due to deficiencies of the three elements are beginning to appear, especially in the absence of K, which is to be expected on such a soil. The groups containing all plots receiving N, P, or K, however, gave yields significantly greater than the NIL plots, and the K plots gave a response over the others (the NP and NIL plots). The group of all plots receiving any fertilizer also gave a response over no fertilizer.

TABLE 4

Plots	NIL	NPK	NP	NK	PK	*v.* all others	Plots	NIL	*v.* all others
NIL	—					2 %	All N plots	5 %	ns
NPK	5 %	—				ns	All P plots	5 %	ns
NP	ns	ns	—			ns	All K plots	1 %	2 %
NK	ns	ns	ns	—		ns			
PK	ns	ns	ns	ns	—	ns			

SUMMARY

A fertilizer experiment carried out on lettuce in soil cultures, partly by the triangle method, is described. It is shown that mixtures of fertilizers containing high nitrogen gave the best response with the soil used. Nitrogen alone, but not phosphate or potash alone, gave a response; mixtures of nitrogen and phosphate, nitrogen and potash, and phosphate and potash, also gave increased yields. In some parallel field trials carried out on the same soil, the complete fertilizer resulted in a significant increase; all plots receiving fertilizer of any kind also gave a response over no fertilizer; the groups of plots receiving nitrogen, phosphate, and potash, gave a significant response over the NIL plot; and all plots receiving potash gave a response over all others.

I thank Mr T. W. McKean for his valuable help in the routine of these experiments.

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PRINCIPAL DECAYS OF BRITISH HARDWOODS

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(With Plates 2–6)

In previous publications the authors (1936, 1938) brought together the available information about the rots of English oak and the principal decays of softwoods used in Great Britain. In this paper the principal decays of the British hardwoods other than those affecting oak are described. While the standardized form of the descriptions of the fungi and of the rots which they cause has been maintained, other aspects, particularly practical and economic recommendations, have been dealt with more briefly. The staining of hardwoods by fungi is not referred to here, since comparatively little work has been done in this country either on the fungi which cause the staining or on the conditions under which staining occurs.

Table 1. *Resistance of various hardwoods to decay*

Timber	Av. loss in dry weight (as % of original dry weight) caused by test fungi after 4 months at 22° C.						Class of durability
	Merulius lacrymans	*Coniophora cerebella*	*Polystictus versicolor*	*Polystictus sanguineus*	*Lenzites trabea*	*Poria vaillantii*	
Acer pseudoplatanus L.	26·6	20·8	39·8	—	22·2	—	Perishable
Aesculus hippocastanum L.	33·6	29·9	32·8	25·9	24·3	27·3	Perishable
Carpinus betulus L.	21·4	11·8	33·2	38·5	20·1	9·4	Perishable
Castanea sativa Mill.	—	—	7·4	—	—	—	Resistant
Fagus sylvatica L.	26·2	30·9	37·7	—	38·6	—	Not resistant
	39·5	36·5	36·6	—	37·6	—	Not resistant
Ilex aequifolium L.	Negligible	3·0	25·2	24·1	6·1	Negligible	Not resistant
Juglans regia L.	4·3	—	9·6	4·5	—	9·3	Moderately resistant
Ulmus hollandica Miller	16·0	—	28·7	23·1	30·4	17·2	Not resistant
Ulmus procera Salisb.	(2·4)	—	22·4	17·2	5·8	1·9	Not resistant
Pyrus torminalis Ehrh.	11·9	24·1	40·2	—	30·8	—	Perishable

Decay in hardwoods

The fungi which decay the timber of broad-leaved trees may conveniently be classified into two groups: those which attack standing trees causing heart rots, and those which are purely saprophytic and which attack felled timber and wood actually in service. The distinction is not absolute, as some fungi of the first group may be found growing saprophytically in fallen timber; however, in the main those fungi which cause important heart rots do not grow as readily in fallen timber as the typical saprophytic wood-rotting species.

Since oak, one of the best known constructional hardwoods, is highly resistant to fungal attack, there is in the trade a sort of general impression that hardwoods are, as a class, more durable than softwoods, and many contracts stipulate merely that a hardwood shall be used in situations where there is a risk of decay, the intention being that oak or similar timber should be used. Actually, many of the so-called hardwoods are readily attacked by

fungi and are no more durable than the wood of the common coniferous trees. The resistance to decay of some of the home-grown hardwoods has been measured by exposing small selected samples to fungal attack under controlled conditions (Findlay, 1938*a*). The results obtained are set out in Table 1, which shows that many of the woods tested were severely decayed by the test fungi in a comparatively short time.

Principal decays of broad-leaved trees

Below are listed some of the most prevalent fungi which cause decay in standing trees of the common British broad-leaved species. They are listed under each tree approximately in the order of their importance. The honey fungus, *Armillaria mellea*, attacks practically all dicotyledonous trees and is not, therefore, included in the following lists. In some situations it is the most prevalent cause of decay, but its virulence seems to depend more on the environmental conditions than on the species of tree involved. *Fomes annosus*, similarly, may attack a wide range of hosts, but it is much less common on broad-leaved than on coniferous hosts. The rots caused by these fungi have been described by the authors (1938).

Alder—*Alnus glutinosa* Gaert.

Alder wood is not resistant to fungal decay. *Polyporus radiatus* is the predominant species, and is responsible for most of the top rot of standing alder trees.

Ash—*Fraxinus excelsior* L.

Well-grown plantation ash is rarely affected by heart rot, though young trees are sometimes severely damaged by cankers which distort the stems. Old hedgerow trees, which are liable to lose large branches and thereby suffer severe wounds, are seldom completely sound. The timber is not resistant to fungal decay. The centre of older trees is often dark brown, but this so-called 'black heart', which is probably due to a kind of heartwood formation, has not been found to be associated with any wood-rotting fungi. *Polyporus hispidus* is by far the most common cause of heart rot in the upper part of the trunk. *Daldinia concentrica* occurs mainly on branches, but works back into the larger limbs and trunk. *Pholiota squarrosa* causes a butt rot. *Fomes fraxineus* causes a basal rot, but is comparatively rare. (See Table 2 for diagnosis of rots of ash.)

Acacia, false—*Robinia pseudacacia* L.

The timber is resistant to fungal decay, and not many fungi have been recorded as causing a rot in it. *Fomes fraxineus* has been collected at the base of decayed trees on several occasions.

Beech—*Fagus sylvatica* L.

The timber of beech is not resistant to decay, and beech trees over a certain age are very susceptible to heart rot. Parkland trees with large branches which are liable to break off in gales are especially prone to infection by fungi such as *Ganoderma applanatum*. In parts of Scotland *Fomes fomentarius* takes the place of *Ganoderma applanatum*, but in England this fungus is absent or very rare. Damage to the roots from rodents leads to infection by fungi such as *Armillaria mellea* and *Polyporus giganteus*, while wounds to the base of the trunk resulting from fires or from felling operations pave the way for infection

by *Ustulina vulgaris*. Decay of the smaller branches is most frequently caused by *Armillaria mucida*, though there are many other fungi such as *Pholiota adiposa*, *Polystictus versicolor*, etc., which may be found growing on dead branches. If beech logs are left lying in the woods after felling they are liable, especially in warm weather, to deteriorate rapidly, the wood becoming affected with incipient decay ('dote'). This decay may be caused by a number of different saprophytic fungi, the most common being *Stereum purpureum*, *S. rugosum*, *S. hirsutum*, *Polystictus versicolor*, *Polyporus adustus*, *Trametes gibbosa* and *Hypoxylon* spp. It is most important that beech logs should be removed from the forest soon after they are felled, if this trouble is to be avoided. (See Table 3 for diagnosis of fungi on standing beech and Table 6 for those on the felled timber.)

Birch—*Betula* spp.

Birch wood is readily attacked by fungi, and the presence of large branch wounds usually leads sooner or later to the development of heart rot. In England, *Polyporus betulinus* is by far the most common parasite, but in parts of the Scottish Highlands *Fomes fomentarius* is the principal cause of heart rot. A form of *Poria obliqua* has also been found on living birch in Scotland, but its prevalence is unknown (see p. 245). Fallen logs of birch rapidly decay unless they are converted soon after felling or stacked so that the surface can dry rapidly. In addition to the common saprophytes, *Trametes rubescens* is fairly common on birch logs, but it is doubtful whether this fungus can act as a parasite in growing trees. (Pl. 4, fig. *c*.)

Cherry—*Prunus avium* L.

Polyporus sulphureus and *Fomes pomaceus* have been found on several occasions causing heart rot in wild cherry trees.

Chestnut, horse—*Aesculus hippocastanum* L.

The timber of the horse chestnut is not resistant to decay, but in spite of this, horse-chestnut trees are not as frequently affected by heart rot as are, for instance, elms, in which the timber is actually more resistant. The fungi which have been found most often on this tree include *Polyporus squamosus* and *Ganoderma applanatum*.

Chestnut, sweet—*Castanea sativa* Mill.

The wood is resistant to decay, and the trees rarely suffer from heart rot. The timber is attacked by a similar range of fungi as oak (p. 222).

Elm—*Ulmus* spp.

Elm wood is moderately resistant to certain fungi, but is readily attacked by other species. The elm is notoriously prone to heart rot, and it is not unusual for affected trees suddenly to collapse or to shed large branches even in calm weather. This is more likely to happen with the common elm than with the Wych or Dutch varieties, since the latter do not normally develop such heavy overhanging branches. *Polyporus squamosus* is by far the most common cause of decay in the upper part of the trunks of elms. *Fomes ulmarius* which occurs always at the base of the tree appears to be specially common in parkland trees which have been damaged by deer. *Ustulina vulgaris* is probably a more common cause of butt rot in elm than has generally been realized. *Pleurotus ulmarius* is not uncommon on the larger branches (see Pl. 4, fig. *b*). *Collybia velutipes* is common on fallen

logs, but is doubtfully parasitic. *Ganoderma applanatum* is not infrequently found on elm, but does not appear to cause such an active rot therein as it does in beech. *Fomes fraxineus* has occasionally been found causing a butt rot. *Pholiota aegerita* is not uncommon at the base of old elm trees. (See Table 4 for diagnosis of fungi causing decay in elm.)

Lime—*Tilia vulgaris* Hayne.

Lime does not appear to be particularly prone to heart rot, possibly owing to the general absence of large limbs on most of the trees in this country, for the timber is not resistant to decay. Severe butt rot caused by *Ustulina vulgaris* has been observed on several occasions.

Oak—*Quercus robur* L. and *Q. petraea* Liebl.

The fungi which attack oak (see Cartwright & Findlay, 1936) include the following: *Polyporus sulphureus*, the principal cause of branch and trunk rot in old parkland trees. *Fistulina hepatica*, the cause of 'brown oak', which in the last stage of attack brings about a hard, brown, cubical rot. *Polyporus dryadeus* attacks the base of old trees causing a white spongy rot. *Stereum spadiceum* causes white- or yellow-piped rot. *Stereum frustulosum*—the cause of a white pocket rot in branches and upper part of the trunk, which gives rise to the condition known as 'partridge wood'.

Pear and apple—*Pyrus* spp.

The woods of these species are probably moderately resistant to decay, but no tests in the laboratory have been made on them. *Stereum purpureum* is less common on apples than on plums, but is capable of causing 'silver-leaf disease' in apple trees that have been drastically pruned. *Polyporus hispidus* is probably the most common cause of heart rot in old apple trees. *Nectria* spp. are responsible for extensive cankering of young shoots.

Plum—*Prunus* spp.

In old orchards *Stereum purpureum* may cause serious havoc, but silver-leaf disease does not appear to affect wild plums so seriously as cultivated ones. *Fomes pomaceus* is very common on old plum trees, and is responsible for most of the rot in hedgerow and neglected orchard trees.

Poplar—*Populus* sp.

Poplar wood is readily attacked by a wide range of fungi, and the trees are liable to canker. *Ganoderma applanatum* is probably the most frequent cause of rot in the standing trees. *Pholiota heteroclita* is also prevalent (for photograph of culture see Pl. 2, fig. *a*).

Sycamore—*Acer pseudoplatanus* L.

The wood is very perishable and deteriorates rapidly if logs are left lying about before they are sawn. Decay in the standing trees is caused mostly by *Polyporus squamosus*, *Ganoderma applanatum* and (less commonly) by *Polyporus hispidus*.

Walnut—*Juglans regia* L.

The timber is moderately resistant to decay, but old walnut trees are frequently badly rotted. *Polyporus squamosus* is probably the most common cause of heart rot in this tree. *Ganoderma applanatum*, *Polyporus hispidus* and *P. sulphureus* also attack it.

Willow—*Salix* spp.

Willow is readily attacked by a number of fungi and the timber is not resistant to decay. Cricket bat willow is liable to a bacterial disease (*Bacterium salicis* Day) which causes a brown discoloration in the wood. The habit of pollarding willows to provide a supply of witheys lays the trunk open to infection. The most common species of wood-rotting fungi on willow are *Ganoderma applanatum*, *Fomes igniarius*, *F. connatus*, *Trametes suaveolens* and *T. rubescens*, the last named being found mainly on fallen branches and logs.

The decay of hardwoods after felling

As soon as a tree is felled the exposed ends of the log become liable to infection by a wide range of saprophytic fungi. The bark continues for some time to afford some protection against the entry of rotting organisms, the length of time depending on its thickness and durability and on its liability to insect attack. Perishable woods such as birch, beech and sycamore may become infected in a few weeks if left lying in the woods during the warmer part of the year, and it is desirable that such timbers should be sawn as soon as possible after felling. The practical aspect of this problem and methods of delaying the deterioration of felled logs have been discussed by Findlay (1938*b*).

On beech and similar timbers the first fungi to make their appearance are usually *Stereum purpureum*, *S. rugosum* and *S. hirsutum*, and on the bark *Bulgaria inquinans*, while later *Polystictus versicolor*, *Polyporus adustus*, *Trametes gibbosa* or *Polyporus tephroleucus*, etc., complete the destruction. As it is impossible here to include descriptions of all these fungi and of the decays which they cause, it has been decided to include a full description only of *Polystictus versicolor* which is probably the most important species, and to summarize in tabular form the outstanding characteristics of the other important species which cause deterioration in felled and stored hardwoods. In these tables attention has been given only to those features which are considered most useful for diagnostic purposes.

Descriptions of principal decays of hardwoods and of the causal fungi

Armillaria mucida (Schrad.) Fr.

Syn. *Mucidula mucida* (Schrad.) Pat.

Occurrence. *Armillaria mucida* is common and widely distributed in Europe and North America. It is almost confined to the beech but has been reported as occasionally occurring on maple, oak, birch and horse chestnut. It is usually found on the smaller branches, but sometimes the fruit bodies appear on dead parts of the trunk.

Fruit body. The fruit bodies of this agaric which usually occur in clusters are very characteristic and cannot be mistaken for those of any other species (see Pl. 4, fig. *e*). The hemispherical cap, 3–8 cm. diam., is generally white but is often greyish, at first, becoming paler as it expands. It is always extremely viscid. The flesh is thin and mucilaginous and the widely spaced white gills are decurrent with a tooth-like projection. The stem, 4–8 cm. long, has a well-developed white ring; it is thickened somewhat at the base which is covered with dark scales. The round spores measure 15–17 μ across (Rea). The fruit body is edible.

Gross characters of the rot. The fungus causes a yellowish white rot, but does not decompose the wood rapidly. It appears to grow more readily on branch wood than on mature wood from the trunk. Orange-coloured zones are always produced in the decayed wood, and the presence of these is a useful means of identifying the rot.

Microscopic details of the fungus in the wood. Hyphae are numerous throughout the tissues of the wood, often being massed in the vessels. They measure 2–5 μ (occasionally up to 8 μ) across and bear numerous clamp connexions. They are hyaline or golden brown. The zone lines are formed

TABLE 2. *Diagnosis of fungi on ash*

Type of rot		Microscopic details of rot			Culture						
Position	Appearance	Clamps	Penetration of walls	Special hyphae or spores	Texture	Colour	Microscopic characters	Oxidase reaction	Optimum temp. for growth °C.	Smell (according to Badcock (1939))	Fungus
Trunk and branches	Yellowish with dark invasion zone, finally spongy	None	Through walls, holes often 3–4 times width of hyphae	Not seen	Thick, plush-like, broadly zoned	Rich yellow to yellowish brown	No clamps	+	30	...	*Polyporus hispidus*
Branches, sometimes trunk	Blotchy white with black specks and lines	None	Through pits and walls	Dark brown hyphae dichotomously branched in vessels	Powdery granular	Greenish black	Conidia on short side branches	– ?	...	Faint, sickly sweet	*Daldinia concentrica*
Butt	White, wood becomes brittle	Numerous	Through walls, large-bore holes	Chlamydospores present in vessels	Smooth, very tough, often with pore surface	Creamy then pale fawn	Clamps present on young hyphae, old hyphae mainly thick-walled, chlamydospores on submerged hyphae	+	26	Faintly fishy	*Fomes fraxineus*
Butt	White, flaky to spongy, usually with dark zone lines	Absent or scarce	Through walls, holes remain small	Zone lines of swollen cells like tyloses, also gumming	Tough skin with rhizomorphs	Pinkish brown	Pseudo-parenchyma of swollen cells	+	25	...	*Armillaria mellea*
Butt	At first yellowish brown then paler, rather stringy	Present	Through walls, very small holes	Some yellow-brown hyphae	Smooth, felted, often fructifies (see Pl. 2, fig. *f*)	Dull orange evenly coloured	Clamps present	+	...	Strong, earthy at 4 weeks	*Pholiota squarrosa*

TABLE 3. *Diagnosis of fungi on beech*

Type of rot		Microscopic details of rot			Culture						
Position	Appearance	Clamps	Penetration of walls	Special hyphae or spores	Texture	Colour	Microscopic characters	Oxidase reaction	Optimum temp. for growth °C.	Smell	Fungus
Main trunk and branches sometimes basal	At first white mottled with brown invasion zone, later white and spongy	Present	Freely through walls; holes fine at first, enlarging later	...	Smooth, chalky, tough	White then *marguerite yellow* or pale fawn†	Aerial hyphae mainly thick-walled, clamps on young hyphae	+	About 30	Faintly of tallow when extracted*	*Ganoderma applanatum*
Main trunk and branches	Yellowish mottling with dark zone lines	Fairly frequent	Through walls; holes at first fine, later enlarging up to 4 μ	Fibrous thick-walled occur, some coloured hyphae	Smooth, even very tough	Pinkish brown changing to dark brown	Aerial hyphae thick-walled and brown, young hyphae with clamps	+	30	Oily, rather like tallow when extracted	*Fomes fomentarius* (in Scotland only)

Branches and trunk	White, flaky	Numerous	Through walls with cylindrical holes 1–2 μ wide	...	Dense, woolly felted, finally tough, often with fructifications	White	Clamp connexions present	+	27	Slightly fragrant at 6 weeks	*Pleurotus ostreatus*
Branches, occasionally trunk	Yellowish white with orange-coloured zone lines	Numerous	Through walls; holes 2–5 μ enlarging sometimes to 7 μ across	Some golden brown hyphae occur	Soft, cotton woolly	White, later with patches of orange brown	Hyphae mostly fine 1–4 μ, occasional clamps	+	23	...	*Armillaria mucida*
Trunk and branches	Brown, cubical with mycelial sheets in cracks	Not seen	Through walls; holes about 5 μ	Swollen hyphae occur especially in rays	Soft, downy, powdery	Salmon pink	Numerous conidia, mostly borne terminally on side branches	–	30	None	*Polyporus sulphureus*
Trunk, especially butt and roots	Whitish rot with narrow black lines, with dark reddish invasion zone	None	Through pits	Dark-coloured hyphae 4 μ across grow out from dark lines	...	White when young then black	...	+	25–30 for germination	None	*Ustulina vulgaris*
Base of trunk and roots	White, spongy	Not seen	Mainly through pits; when through walls only fine holes	Hyphae with swellings observed	Soft at first, cotton woolly later, rather more leathery	White, finally with brown patches	Occasional large hyphae up to 7 μ, large chlamydospores in old cultures	+	...	...	*Polyporus giganteus*

* I.e. when a small portion of the culture is removed from the tube for examination.

† Names of colours printed in italics are according to Ridgway, R., *Color standards and color nomenclature*, 1912.

TABLE 4. *Diagnosis of fungi on elm*

Type of rot		Microscopic details of rot			Culture						
Position	Appearance	Clamps	Penetration of walls	Special hyphae or spores	Texture	Colour	Microscopic characters	Oxidase reaction	Optimum temp. for growth °C.	Smell	Fungus
Upper part of trunk and branches	White, spongy, with sheets of mycelium	Present	Through walls; holes enlarged up to 7 μ	Oval or elongated swellings present	At first loose and cottony, then a dusty, rather brittle skin is formed	White, then pale brown	Numerous oidia formed by fragmentation of hyphae	+	24	...	*Polyporus squamosus*
Branches and trunk	Mottled rot, finally white	Numerous	Through walls; holes enlarged	...	Dense cotton-woolly, felted	White	Numerous clamps; some hyphae with elongated swellings	+	...	...	*Pleurotus ulmarius*
Butt, causing conical rot column	Brown, crumbly, with cracking along and across the grain	Rarely seen	Freely through walls; very fine penetrations	A few thick-walled chlamydospores; larger hyphae may be yellow-brown	Feeble, loose spidery growth; later small round lumps appear, especially on medium with peptone	Colourless; lumps may be fawn	A few chlamydospores noted but no clamps	–	28–30	...	*Fomes ulmarius*
											Ustulina vulgare, see under beech (Table 3)

TABLE 5. *Diagnosis of fungi on willow*

Type of rot		Microscopic details of rot			Culture						
Position	Appearance	Clamps	Penetration of walls	Special hyphae or spores	Texture	Colour	Microscopic characters	Oxidase reaction	Optimum temp. for growth °C.	Smell	Fungus
Main trunk	Early stage yellowish white, area bounded by dark zone; later soft white rot with concentric zone lines	Not seen	Through walls, forming large bore-holes	Zone lines consist of thick dark gnarled hyphae	Thick felted velvety, even mat	*Yellow ochre* to *buckthorn brown*	Hyphae mostly coloured, and thick-walled, 2 μ wide	+	28-30	Some strains smell of methyl salicylate	*Fomes igniarius*
Trunk	White	Numerous	Through walls; holes up to 10 μ across	...	Soft, dense felted	White to cream	Secondary spores formed in clusters	+	28	Strongly of anise	*Trametes suaveolens*
Branches and trunk, mostly on dead trees	...	Numerous	Through walls; holes variable in size	A few chlamydospores in vessels	Thick tough felted, tufted at top, usually with pore surface	White then pinkish brown, finally *cinnamon brown*	Fibrous hyphae with very thick walls are plentiful	+	...	Faintly of tallow when extracted	*Trametes rubescens*
Trunk	Soft white rot	Not seen	...	...	Thin compacted cottony mat, usually contaminated with bacteria	White	Sporophores formed in culture bear incrusted cystidia	Faintly +	About 25	...	*Fomes connatus*
											See also *Ganoderma applanatum*, *Armillaria mellea*, etc.

TABLE 6. *Diagnosis of fungi on felled hardwoods*

Type of rot		Microscopic details of rot			Culture						
Timbers most often attacked	Appearance	Clamps	Penetration of walls	Special hyphae or spores	Texture	Colour	Microscopic characters	Oxidase test	Optimum temp. for growth °C.	Smell	Fungus
Most hardwoods	At first a white mottling, then white	Plentiful	Freely through walls, small holes	...	Smooth even tough skin	White, then cream, sometimes with yellowish or brownish patches	Hyphae bear numerous clamps of raised 'hoop' type	+ strong	29	Faintly of fish and tallow, when extracted	*Polystictus versicolor*
Most hardwoods	At first a white flecking, then white	Present	Through walls, bore-holes become enlarged	...	Smooth even soft, cotton-woolly	White, slight yellowing on ageing	A few swellings of chlamydospore type, clamps present	+	25–27	Faintly fragrant, terpenoid	*Polyporus adustus*
Most hardwoods, especially oak sapwood	White soft stringy rot, sometimes in pipes	Present	Usually through pits, when through walls holes remain small	Hyphae much branched	At first loose and cottony, later a tough felted skin	White, soon turning yellow	Young hyphae bear 'whorled' clamps	+	25	Faintly fragrant or absent	*Stereum hirsutum*
Beech, birch, plum, etc.	First effect is a brown discoloration, later white mottling	Present	Bore-holes remain fine	...	At first loose and cottony, later soft felted even	White, later slight tinges of fawn	Young hyphae may have whorled clamps	+ but slight	27	Slightly fragrant at 6 weeks	*Stereum purpureum*

Beech, oak, many other woods	Mottled at first, then yellowish with dark lines, rays may be filled with dark material	Presen	Bore-holes cylindrical, 0·5 up to 6 μ across	Orange-brown hyphae occur	Soft, cotton-woolly, inclined to be felted	White, soon turning to yellowish or pinkish buff	Hyphae uniform infrequently septate, clamps scarce	+	20–22	Fruity, like over-ripe bananas	*Stereum rugosum*
Beech and most hardwoods	White rot	Plentiful	Through pits and walls, rays particularly attacked	...	Rather slow growing, even smooth cotton-woolly soft becoming tough (see Pl. 2, fig. *b*)	White, later with brown patches, young fruit bodies pale orange yellow	Most of aerial hyphae thick-walled without clamps, clamps scarce on other hyphae	+	About 30	Fishy, oily when extracted	*Lenzites betulina*
Beech	White	Plentiful	Through pits and walls, holes enlarged	...	Rapid growing, at first loose and cotton-woolly, later mat becomes slightly farinaceous (see Pl. 2, fig. *f*)	White	Mat consists mainly of thick-walled hyphae about 2 μ wide, clamps scarce	+	30	...	*Trametes gibbosa*
Beech and hornbeam	White stringy with chocolate-coloured zone lines	Not seen	Bore-holes fine at first, later enlarging	Dark frequently septate hyphae which are sometimes swollen	Thick woolly mat, inclined to be silky (see Pl. 2, fig. *i*)	Rich yellow-brown tints	Hyphae may aggregate to form strands, long setae but no clamps found	+	30	...	*Polyporus cuticularis*
Beech, many tropical timbers	Slight pale mottling never causes advanced stage of rot	Present	Mainly through pits	...	Soft cotton-woolly, fruit bodies usually form in a few weeks	White, sometimes brownish spore deposit	Clamps present	+ but feeble	30 (max. is over 40)	...	*Schizophyllum commune*
Practically all kinds of wood	Wood much darkened and split mainly along the grain, dark strands usually present	Present	Freely through walls, holes average 3·5 μ across	Some of wider hyphae constricted at septa	Soft, like fine flannel, often with many fine strands	*Maize yellow* darkening to *snuff brown* or *tawny olive*	Hyphae at edge of young culture have whorled clamps (up to 5 at a septum)	–	23	...	*Coniophora cerebella*
Many kinds of wood	Brown cubical	Present	Through walls	Hyphae mainly fine	Soft, thin farinaceous to powdery	White, then *pale cartridge buff*	Numerous secondary spores averaging 8 × 10 μ, borne in tufts	–	25	...	*Polyporus fumosus*
Imported hard and softwoods, mainly tropical	Brown cubical	Present	Freely through walls, bore-holes become enlarged	Fibrous mycelium	In mature culture, a profuse growth at the top of slant, with scant growth at bottom	*Orange buff, ochraceous salmon*	Clamps frequent, oval chlamydospores abundant, oidia present on aerial hyphae	–	35	Slightly acrid, rubber and iodine	*Lenzites trabea*

by the massing together of these rather wide golden brown hyphae which are embedded in a yellowish brown gummy material. The hyphae bore through the cell walls forming holes which average at first 2·5 μ across but, later, may enlarge to 7 μ across.

Fungus in culture. The fungus grows readily and moderately quickly on malt agar forming a featureless, soft, cotton-woolly mat which is at first rather thin, but later becomes somewhat denser with smooth patches bounded by tiny ridges of mycelium. The culture remains white with an occasional trace of pale orange brown and the mat is always soft and easily torn. Most of the hyphae are somewhat fine (1–2·5 μ) and only occasionally reach 4 μ across. Clamp connexions occur but are not frequent. No secondary spores have been observed. The fungus grows well on bread and on sterilized wheat grains, and on these media soon produces sporophores which may be typical or consist of an elongated stalk with little or no pileus.

Physiological data. Fischer (1909) found that the spores, when fresh, germinate readily in water and in ordinary nutritive media, but that they do not remain viable in a dry condition for more than a few months. He carried out a number of experiments to determine whether the fungus can be actively parasitic, but his inoculations were all unsuccessful and there appears to be no conclusive evidence on this point. The fungus grows fairly rapidly in culture, the daily increment of a colony on 2% malt agar being about 8 mm. at the optimum temperature which is about 23° C. The fungus grows comparatively quickly at low temperatures.

Economic importance. While *A. mucida* is exceedingly common in all beech woods and brings about the decay of much branch wood there is no evidence to show that it can cause a heart rot in actively growing trees. Where it is found growing in the trunk, its attack is usually confined to the neighbourhood of some large superficial wound, and it is rare to find large-sized logs rotted by this fungus. As soon as the sapwood is killed it rapidly becomes liable to infection with this fungus, and any suppressed lower branches which are damaged soon develop a crop of sporophores of *A. mucida*. At present, therefore, it cannot be considered a serious parasite. On ornamental trees any branches infected with the fungus should be cut off cleanly close to the trunk and the wound dressed with a preservative.

Fomes fomentarius (Linn.) Fr.—tinder fungus

Syn. *Ungulina fomentaria* (Linn.) Pat. *Fomes nigricans* Fr.
German: *Zünderschwamm*; French: *Amadouvier*

Occurrence. Fomes fomentarius is widely distributed in Europe and America, and it has been reported from India and Australia. In England it is very rare but is common in parts of Scotland, where it is often the most common species occurring on the birch. Most of the earlier references to this fungus on the beech in England probably refer to *Ganoderma applanatum. Fomes fomentarius* occurs on living trees and on stumps, and is able to continue its growth on dead felled timber so long as this remains damp, but probably only rarely infects *de novo* fallen logs. It occurs on a wide range of leafy trees but is most common on birch and beech. Specimens with a dark shiny crust have sometimes been described as a variety—*nigrescens* of Klotsch and sometimes as another species, *F. nigricans* Fr.—but Macdonald (1938) has shown that the dark form occurs alongside the silvery grey form and that there is no justification for considering the dark form as a separate variety.

Sporophore. The fruit body is of the typical hoof-shaped perennial *Fomes* type and may sometimes attain a very large size, reaching a width of up to 40 cm. (see Pl. 3, fig. *f*). The crust is smooth, thick and hard and concentrically zoned; its colour varies from grey or greyish brown to almost black. The thick flesh is punky to soft corky and dull brown. The tubes are long and distinctly stratified, the pores are extremely small (3 to the mm.) and the pore surface powdery grey. The oblong colourless spores measure 15–18 × 5 μ (Rea) and afford a ready means of distinguishing between this species and *Ganoderma applanatum*. There are no cystidia. Buchwald (1938) states that this fungus produces an exceptionally large number of spores, one fruit body which he examined producing 887 million spores per hour corresponding to a rate of 139 millions per sq. cm. per day. He estimated the weight of an individual spore as just over 1×10^{-11} g., since 1 g. of spores was estimated to contain 9 milliards of separate spores.

The rot. Decay is usually confined to the upper parts of the trees, the fungus gaining entrance through broken branches and other wounds in the stem. It causes a mottled yellowish white rot which shows narrow dark zone lines running through it. The early stage of the rot is bounded by a narrow brown invasion zone. Sheets of pale yellowish white mycelium may be found filling the

cracks in the decayed wood. It attacks both the heartwood and the sapwood. Lohwag (1931) described a 'furrowing' of beech trees, resulting from infection by *Fomes fomentarius* which causes longitudinal grooves to develop in the heartwood, along which decay proceeds rapidly. When the groove reaches the cambium, the latter is killed, but in the adjacent regions cambial activity is increased so that the grooves are lined with healing overgrowth.

Microscopic details of the rot. Hyphae are moderately plentiful in decayed wood, particularly in the medullary rays, which are the first elements of the wood to be attacked. They are of two kinds, first, rather fine hyaline hyphae averaging about 2·5 μ across, which penetrate the cell walls, and bore holes that are at first very fine but which later are enlarged up to about 4 μ, and secondly, fibrous thick-walled hyphae, colourless or golden brown, which are usually markedly constricted when passing through the cell walls. In places these coloured hyphae are much swollen and form a sort of pseudoparenchyma which may plug the vessels. Clamp connexions are fairly frequent on the hyphae in the wood.

Fungus in culture. The growth and appearance of the fungus in culture have been fully described by Fritz (1923), and it has been found that cultures made from sporophores collected in Scotland closely resemble isolations received from Canada. On malt agar growth begins with fine, radiating, appressed, colourless hyphae and a closely woven, tough, smooth, even mat is soon formed. After about a week in cultures grown in the light, the colour may be *light ochraceous salmon*, but it soon darkens to *Mikado brown* or *avellaneous*, finally becoming *snuff brown* or *argus brown*. When the mat is very old it becomes papery and brittle. Cultures grown in the dark do not develop nearly so much colour as those in the light. The young hyphae, which bear clamp connexions, are 2–5 μ wide and colourless; the mature mat consists mainly of dark brown fibrous hyphae without clamp connexions. The submerged mycelium consists of much-branched, rather wide colourless hyphae (4–5 μ), some of which are heavily encrusted with crystals, and of much-branched fibrous threads 1–2 μ wide, which have very thick walls. A few small oval chlamydospores are occasionally seen on the submerged hyphae, and crystals are formed freely in the medium. Some of the wider aerial hyphae in old cultures break up into oidia about 15–20 μ long.

Physiological data. The rate of growth on malt agar is rapid, a boiling tube slope being covered in about 14 days at laboratory temperature. The rates of growth at different temperatures have been determined in the manner previously described by the authors (1934) and were as follows:

Temp. °C.	10	16	20	22	24	28	30	34	38
Av. daily increment, mm.	4·1	7·2	10·4	13·0	15·0	16·4	17·0	11·6	0

The fungus was thus capable of growing over a wide range of temperatures and had an optimum for its growth at about 30° C. Macdonald (1938) found that when the fungus is grown on a medium containing tannic acid brown haloes are produced, indicating the production of oxidase.

Economic importance. On the Continent *F. fomentarius* is regarded as one of the most important heart-rotting fungi of the beech, and in certain parts of Scotland it is the cause of much top rot in the birch as well as in the beech. Control of the disease depends on the avoidance of wounds.

Fomes fraxineus (Bull.) Fr.

Syn. *Ungulina fraxinea* (Bull.) B. & G. *Polyporus cytisinus* Berk. *Polyporus incanus* Quel.

Occurrence. *Fomes fraxineus* is widely distributed in Europe and North America; in this country it is comparatively rare. It attacks a number of broad-leaved trees including ash, false acacia (*Robinia pseudacacia*) and laburnum, and it has also been found by the authors on elm, poplar and beech. It is always found near the base of the tree in which it causes a butt rot.

Sporophore. The fruit body is a thick, woody bracket which sometimes reaches 40 cm. across, and may be imbricated. The upper surface is at first a cream colour, later becoming isabelline and finally dark brown or blackish. The pale yellowish or pale fawn flesh is soft at first but soon becomes hard and woody. The tubes which are concolorous with the flesh are usually stratified and 5 -25 mm. in length; the orifices of the pores are small (4 to the mm.) and pinkish brown. The subglobose spores are colourless and measure on the average 7 × 6 μ. The fruit body resembles that of *Fomes ulmarius*, but is generally more flattened and the pore layer is never the bright reddish brown which is characteristic of *F. ulmarius* (see Pl. 3, fig. *e*).

The rot. The fungus causes an active rot in standing and felled trees; the decayed wood in the final stage is whitish. Bourdot & Galzin (1927) recorded an instance in which a cavity about 5 ft.

long and 16 in. across was formed in an oak log, and where all wood had been replaced by a felt-like mass of light-coloured mycelium. In an early stage of decay the wood becomes 'brash' and the grain breaks off 'short' under strain.

Microscopic details of the rot. The colourless hyphae penetrate the cell walls freely, forming fairly large bore-holes which tend to be hour-glass-shaped and only utilizing the pits in the medullary rays and wood parenchyma to pass from one cell to another. The hyphae in wood vary from 1 to 7 μ diam., but are usually about 2 μ; they bear numerous clamp connexions. Chlamydospores 4 × 5 μ to 12 × 15 μ in size occur in the vessels, and appear to be produced most frequently when conditions are either too dry or too moist for the hyphae to flourish.

Fungus in culture. The growth and appearance of the fungus in culture on 2% malt agar were described by Montgomery (1936*a*) and by W. A. Campbell (1938). Growth starts from the transplant with fine, appressed, radiating hyphae, and gradually becomes thicker so that a close, flat, tough, felted mat is produced; eventually, especially in the dark, this may form a fairly thick cushion of tough mycelium at the base of the slope. In the light a pore surface originating in small depressions at the upper end of the slope, usually develops after about 3 weeks. Delicate tints of *cartridge buff* and *cream buff* can be observed in young cultures; in old cultures the colour may fade out and the cultures become a dirty creamy white. The young hyphae are 2–5 μ diam. and bear clamp connexions; in a mature culture the majority of the aerial hyphae are 2 μ wide, have thick hyaline walls and contain few septa. At the upper end of the slope a much-branched fine mycelium of rather coralloid appearance may be formed. The submerged hyphae are much branched, frequently septate, and bear numerous clamp connexions. Many chlamydospores are present in the submerged hyphae; these average 13 × 10 μ. Montgomery (1936*a*) showed that normal basidiospores are produced on the hymenium formed in culture. These spores germinated readily within 24 hr. and gave rise to haploid cultures. Crossings of these cultures showed the fungus to be heterothallic and indicated the presence of a two-factor basis of the sex determination.

Physiological data. The following data have been summarized from Montgomery's paper (1936*a*). The rate of growth on malt agar is moderately rapid, the daily increment in diameter of Petri dish cultures being 8·6 mm. per day at the optimum temperature which is 26° C. The maximum inhibiting temperature is about 33° C. The resistance of the fungus in culture to heat was examined, and it was found that it was killed after 4 hr. exposure to 60° C. The most rapid growth took place in a malt medium with an initial *p*H of 6·0: the *p*H of a medium adjusted initially to *p*H 5·5 dropped after 2 weeks and then rose to 7–8. The enzymes extracted from powdered mycelium grown in liquid culture include oxidizing enzymes in addition to diastase and emulsin. The fungus was able to cause considerable loss in weight in ash wood, the rate of loss during the first stages of attack being much higher in sapwood than in wood from the centre of a tree, probably owing to the rapid destruction of cell contents which were present only in the sapwood.

Economic importance. As the fungus is comparatively rare in England it cannot be considered of great economic importance, but where present it may cause extensive heart rotting.

Daldinia concentrica (Bolt.) Ces. & de Not.

Occurrence. According to Tulasne (1863) this fungus grows all over the world. In this country it is very common on ash, but in France it is stated to be most frequent on alder and also to grow freely on walnut and birch.

Sporophore. The fruit bodies consist of practically smooth, hemispherical black lumps 3–4 cm. or more in diameter. These lumps, which have when mature the consistency and appearance of charcoal, are concentrically zoned. The perithecia occur just below the surface, and from these very narrow canals lead to the tiny ostioles on the surface. The narrow cylindrical asci contain eight black spores which average 13 × 6·5 μ. The young fruit body is covered with conidia borne on branched conidiophores like a *Botrytis*. The conidia are fawn in mass (appear ovate and colourless under the microscope); they measure 6·5–8 × 5·6–5 μ (Tulasne). When wood infected with *Daldinia concentrica* is moistened and kept in a damp atmosphere, bunches of conidiophores develop.

Gross characters of the rot. In ash the fungus causes a white, mottled rot, in which black specks or dark lines embedded in the wood can be seen. Panisset (1929) described how the type of ash known as 'calico wood' is caused by this fungus. The black specking is due to the large vessels of the spring wood being filled with dark-coloured mycelium.

Microscopic details of the rot. Panisset described three types of hyphae in the diseased wood: (1) Very fine, colourless hyphae which permeate all the elements of the wood, passing through the

cell walls by means of the pits. The hyphae also penetrate the cell walls, being much constricted in their passage through the fine bore-holes. (2) Wider brown hyphae, which in the larger vessels are dichotomously branched, the tips of the ultimate branches being pointed. (3) Irregularly and sparsely branched brown hyphae in the ray cells.

In the later stages of attack brown, amorphous, granular masses appear, especially in the fibres and vessels, and the colour of the hyphae darkens to almost black. Panisset suggests that the attack on the cell walls takes place first via the pits on the middle lamella surrounding the pit membrane.

Fungus in culture. A powdery, soft mat is produced on malt agar. The colour of this varies according to the vigour of the culture. Cultures from fresh isolations appear greenish black and granular when mature, but after repeated subculturings the culture may appear mainly a dirty fawn. The medium always becomes stained a dark brown. In addition to ordinary hyaline hyphae, dark dichotomously branched hyphae develop in cultures; these sometimes become aggregated into small spherical bodies about 300 μ across. Typical conidia, borne in bunches on short side branches, are developed in culture on agar.

Physiological data. The ascospores and conidia germinate readily in water and in slightly alkaline as well as in very slightly acid solutions. Panisset found that the fungus will grow on a number of different carbohydrates but that it does not decompose lignified material very actively. No reaction is obtained when guaiacum solution is added to a liquid culture, so oxidase production must be feeble or lacking.

Economic importance. Probably *Daldinia* is not actively parasitic and attacks only those parts of the tree which are already moribund or dead. Once established in a dead branch, it sometimes appears to be able to work back into sound living wood. It can attack only through wounds, broken branches, stubs, etc., and does not seem to spread very rapidly.

Fomes igniarius (Linn.) Fr.—false tinder fungus

Syn. *Phellinus igniarius* (Linn.) Pat.

Fomes igniarius is common and widely distributed in Europe and America and has been reported from India and Australia. In this country it is more or less confined to willow, but is occasionally found on ash (Rea); abroad it has been reported on a wide range of broad-leaved trees but everywhere is found most frequently on Salicaceae. A form occurring on birch has been described as *Polyporus nigricans*, and there has been much confusion between this plant and certain forms of *Fomes fomentarius*, although they are readily distinguishable on the basis of the spores alone. Verrall (1937) showed that there is great variation in the general appearance of sporophores of *F. igniarius*, and that in America these fall into three fairly distinct groups, the aspen type, the white birch type and the type from miscellaneous hosts.

Sporophore. The fruit body is hoof-shaped or expanded into a thick broadly attached bracket which may be up to 20 cm. across. The recently formed margin is fawn, the older part being a greyish black and usually much cracked and split. The dark brown flesh is extremely hard. The stratified tubes are 2–8 mm. long, the pores are very fine (4 to the mm.), and their mouths are yellowish or greyish brown. The hyaline, subglobose spores measure 5–6 × 4–5 μ (Bourdot & Galzin). Dark-coloured pointed setae about 20 μ long are present, though sometimes rather scarce. It has been suggested by several writers that the dark sterile abortive sporophores which resemble cracked pieces of coal and which sometimes occur on living birches, belong to *F. igniarius*, but Campbell & Davidson (1938) concluded as a result of a cultural study that the sterile fungus is a species of *Poria*, probably *P. obliqua* (see p. 245), and this has been confirmed by Findlay (1939).

The rot—white heart rot. Descriptions of the typical rot brought about by this fungus have been given by a number of writers from Hartig (1894) onwards. Lindroth (1904) described a decay in birch caused by *Polyporus nigricans*, and there is little doubt that he was dealing with the dark variety of *Fomes igniarius*. A full account with excellent photographs of the white heart rot caused by this fungus and by the *nigricans* variety were given by Schrenk & Spaulding (1909). Rudau (1917) gave a lengthy description of the effect of *Polyporus igniarius* upon a number of broad-leaved European trees and listed the hosts upon which it had been recorded. Hubert (1931) described the different stages of the rot as follows. The early stage of decay as seen at the end of a log appears as an irregularly shaped yellowish white area in the heartwood; this area is usually bounded by the invasion zone, a yellowish green to brownish black band. As decay progresses, the wood becomes soft and whitish, but shows few, if any, cracks. At this stage numerous fine, irregular concentrically arranged,

black lines may be found embedded in the whitish, spongy, rotted wood. Decay is more or less confined to the heartwood, but the fungus can sometimes spread into and attack living sapwood. Rot may occur in any part of the bole, but is most often found in the main portion of the trunk.

Microscopic details of the rot. Hyphae are plentiful in an advanced stage of the rot, but have not been found beyond the heavily discoloured invasion zone. The hyphae are at first stout, but in later stages are mainly very fine (about 1 μ) and much branched. They penetrate the walls freely, causing large bore-holes. The zone lines consist of dark thick-walled gnarled hyphae. Clamp connexions have not been observed on the mycelium in the wood.

Fungus in culture. A detailed description of *Fomes igniarius* in culture was first given by Fritz (1923). Verrall (1937) has since shown that there is great variation between different isolates of this fungus, and he distinguishes three main groups which more or less correspond to the three main types of sporophore: (1) a slow-growing type isolated from aspen which produces a strong odour of methyl salicylate; (2) a rapid-growing type isolated from white birch which does not produce methyl salicylate; (3) types from other hosts which resemble (2) in rate of growth, consistency of mat, and in not regularly producing methyl salicylate. W. A. Campbell (1938) described the cultures of typical *F. igniarius* and of its varieties, and pointed out how they can be distinguished.

The following description is based on cultures made from a sporophore collected from a willow near Oxford. In the light a very thin sparse growth of appressed hyphae develops at first, and, later, a smooth even closely felted almost velvety mat, with only traces of zonation, is formed. This is a uniform *buckthorn brown*. Other tints noted during the development of the culture were *mars yellow, sudan brown—raw sienna—argus brown* and *antique brown*. A cushion or thick bracket of mycelium, paler on top, often develops at the top end of the slope, especially on 5% malt agar. In the dark a thicker mat more varied in colour is produced; in this the general tone is *yellow ochre*. The mat consists of a closely woven tangle of fibrous, thick-walled, yellowish brown hyphae, averaging 2 μ diam.; these are sparingly branched and possess few septa. No clamp connexions have been observed. Submerged in the medium, hyaline much-branched hyphae up to 5 μ diam. occur in addition to the yellow fibrous hyphae. Below the mat or embedded just below the surface of the agar there is sometimes a plate of tissue consisting of pseudoparenchyma built up of 'cells', on the average about 10 μ across, formed by swollen portions of the hyphae. This pseudoparenchyma probably corresponds to the similar plates formed in the wood, which in section appear as 'zone lines'.

Physiological data. The rate of growth of isolates from different hosts varies considerably, but on the whole the fungus grows rather slowly in culture. The rates of growth at different temperatures of a strain isolated from willow are as given below:

Temp. °C.	10	17	22	24	28	30	34	38
Av. daily increment, mm.	2·0	4·2	5·2	5·7	6·4	6·2	4·2	0

The optimum found in these tests was therefore about 28° C., a figure agreeing with that found by Verrall (1937).

Hasenöhrl & Zellner (1922) found that oak wood which had lost 74% of its weight and all its tannin and starch did not differ from sound wood materially in the relative proportion of its principal constituents, indicating that they had all been taken up by the fungus without selective action on any particular constituent, i.e. it causes a white rot in which cellulose, lignin and the pentosans are all attacked. The enzymes of *F. igniarius* were examined by Schmitz (1921), who found a series of hydrolysing enzymes including cellulase and hemicellulase. Verrall (1937) found that the isolates from aspen which normally produce methyl salicylate can tolerate very much higher concentrations of this compound in a medium than can other isolates that do not produce it naturally.

Economic importance. In Great Britain *F. igniarius* is not sufficiently common to be considered a serious parasite, but it might become important if willow and poplar trees were grown more extensively, particularly if they were grown in plantations. On any other broad-leaved trees in this country the fungus is rare and of no economic importance. On the Continent and in America it is of first class importance on a number of broad-leaved trees, but is especially destructive to poplars and birches. Schmitz & Jackson (1927) state that *F. igniarius* may be regarded as by far the most important cause of heart rot of aspen in Minnesota; in trees 70 years old the total amount of rot affects 31·2% of the merchantable volume of the trees.

Prevention of decay by *F. igniarius* is largely a matter of preventing the formation of branch wounds which may allow the fungus to get into the heartwood, and the disease is unlikely to become serious in commercial plantations of aspen grown on fairly short rotations of about 50 years.

Fomes pomaceus (Pers.) Lloyd

Syn. *Phellinus fulvus* (Scop.) Pat. *Fomes fulvus* (Scop.) Gill.

Occurrence. *Fomes pomaceus* is widely distributed in Europe and America and has been reported from Australia. It is common in this country on old plum trees in neglected orchards. As far as is known it is restricted to trees belonging to the order Rosaceae, being found most frequently on *Prunus* spp. It seems probable that the rot of birch, stated by Schrenk & Spaulding (1909) to be due to *Fomes fulvus* (a synonym of *F. pomaceus*), was caused by some other fungus, for they described the rot as a red-brown one.

Sporophore. The fruit body which is hard and woody is, when perfectly formed, a thick bracket usually about 5 cm. across, triangular in section or somewhat hoof-shaped with an obtuse edge (see Pl. 3, fig. *d*). The resupinate or half-resupinate form is, however, more common, and appears as a plate about 1 cm. thick adhering to the bark. The colour of the upper surface when this is present is an ashy grey becoming in the older parts a brownish grey, the pore surface is at first ashen then finally dark cinnamon brown. The tubes, which are a similar colour and 4–6 mm. long, terminate in minute pores (4–5 to the mm.). The colourless spores are almost spherical and measure 6×5–$6 \times 6\,\mu$. The flask-shaped cystidia have pointed ends, are dark brown at the base and somewhat paler at the top, and average $18 \times 8\,\mu$.

Gross characters of the rot. A cross-section of a trunk or branch of a plum tree infected with *F. pomaceus* shows a central decayed portion in which the wood is white and crumbling, surrounded by a dark purplish brown zone of incipient decay 1–2 cm. wide which is hard and firm (Pl. 6, fig. *d*).

Microscopic details of the rot. The microscopic appearance of the diseased wood was described in detail by Fisher (1934). In the zone of incipient decay where the wood appears dark purplish brown a copious deposit of brown gum can be seen filling the vessels; in the later white-rot stage this gum practically all disappears, evidently being destroyed by the fungus. Hyaline hyphae are plentiful in the decayed wood, and in an advanced stage of rot they are mostly very fine (1–$2{\cdot}5\,\mu$ across). Clamp connexions have not been observed. The hyphae penetrate the cell walls freely, causing rather large bore-holes. The larger vessels may be filled with a weft of fine thick-walled rather fibrous, honey-coloured hyphae.

Fungus in culture. A brief description of the fungus in culture was first given by Rumbold (1908). On malt agar growth begins as a downy ball over the transplant and spreads as a thick soft woolly mat, the aerial mycelium practically keeping pace with the appressed. The mat finally becomes very thick woolly and blanket-like, with a somewhat tufted flocculent surface. The mycelial weft may eventually almost fill the culture tube. The mycelium is at first white but colour soon develops, particularly in the light: the first tints observed are *pinkish buff* to *warm buff* then *cinnamon buff* to clay colour and finally *ochraceous tawny* to *tawny olive*. The young advancing colourless hyphae vary from $1{\cdot}5$ to $6\,\mu$ diam. while the older aerial hyphae are mostly $2\,\mu$ across. They soon darken from pale yellowish to a rich yellow brown, the contents sometimes being a dark brown. No clamp connexions have been observed. Cubical or rhomboidal crystals are numerous in the medium of old cultures. The culture generally resembles that of *F. ribis*, from which it differs in the slower development of colour, especially in the dark and in the absence of some of the redder tints.

Physiological data. The following rates of growth at different temperatures were determined by the measurement of Petri dish cultures on malt agar:

Temp. °C.	10	20	22	24	28	30	34	38
Av. daily increment, mm.	1·7	5·3	5·5	6·5	7·4	10·7	3·3	0

The fungus, therefore, grows moderately quickly at the optimum temperature which is about 30° C.

Economic importance. *F. pomaceus* causes a fair amount of damage in old orchards. Old trees having large branches which may break, forming wounds which expose the heartwood, are very liable to attack. Once a tree is attacked it is difficult to check the spread of the rot, therefore control of the disease is a matter of preventing infection. Broken branches should be cut off cleanly and the stub dressed with an antiseptic coating.

Fomes ulmarius (Sow.) Fr.

Syn. *Ungulina ulmaria* (Sow.) Pat.

Occurrence. *Fomes ulmarius* is common in this country, but apparently is rather less frequent on the Continent. It does not appear to have been reported from America or Australia. There has been some discussion as to whether *F. geotropus*, a closely related fungus, is in fact merely a tropical form

of the same species, as C. G. Lloyd (1915) suggested. While the authors have not had the opportunity of examining a range of sporophores of the two fungi, examination of cultures of *F. geotropus* shows that the fungi are by no means identical, but whether the differences are sufficient to warrant the continued separation of the two fungi as distinct species must await further examination. *F. ulmarius* in Great Britain appears to be restricted to the elm, while *F. geotropus* occurs on a number of hosts, particularly on *Cryptomeria* spp. This difference in range of hosts, together with certain physiological differences referred to below, suggests that the two fungi should at least be treated as distinct varieties. The fruit bodies appear at the base of living trees, usually close to the soil, and very rarely higher up the trunk; they also frequently develop on stumps of diseased trees which have been felled. Sometimes they develop inside hollows at the base of old trees. Cases of the fruit bodies occurring in drain pipes have been reported, and Lorrain Smith (1919) described a case in which a huge mass of the mycelium of this fungus completely blocked a sewer.

Sporophore. The thick bracket-shaped, perennial fruit bodies sometimes reach a large size, and may attain a width of 30 cm. or more (see Pl. 3, fig. *c*). The whitish or cream-coloured upper surface is usually lumpy, and sometimes the whole sporophore consists of irregular rounded masses. In old fruit bodies the upper surface is always discoloured and greenish with algal growths. The flesh is white, tough and fibrous when young, later becoming pale buff and very hard and woody. The long stratified tubes are bright cinnamon, which is characteristic of the species and affords a ready means of distinguishing the fungus from the somewhat similar *Fomes fraxineus*, in which the tubes are much paler. The pores are small, 0·25–0·4 mm. across, and the whole pore surface is similar in colour to that of the tubes, but often less brilliant. The spores are colourless, more or less globose, and measure 6–7 μ across (Rea).

Gross characters of the rot. The rot is usually confined to the butt of the tree, in which it causes a conical column of decay. In an advanced stage of rot the wood is dark brown, very friable and tends to split up into brick-shaped pieces by the formation of cracks along and across the grain (see Pl. 5, fig. *b*). No thick mycelial sheets develop in these cracks.

Microscopic details of the rot. Hyphae are fairly plentiful in the decayed wood and may be massed in the vessels, the medullary rays remaining more or less unattacked till a late stage. The hyphae are mostly fine (1·5–2·0 μ diam.), but a few wider ones (up to 6 μ across) occur in the vessels. The majority of the hyphae are hyaline, but some of the larger ones are yellow-brown. Clamp connexions are very rarely observed; a few thick-walled chlamydospores occur. The hyphae penetrate the walls freely, but form only very fine perforations. When the fungus is growing, actively numerous fine side branches may be produced on a main hyphae. These come off at right angles and penetrate the wall directly.

Fungus in culture. The fungus makes rather feeble growth in culture on agar media, and it is only with great difficulty that pure cultures can be obtained from tissue plantings from sporophores. Even cultures made from fresh, active sporophores are almost invariably contaminated with bacteria. Successful isolation of the fungus can sometimes be achieved by tissue plantings in a medium consisting of sawdust to which small quantities of nutrients have been added (Badcock, 1941). Growth is moderately slow on malt agar; at first on this medium only a loose rather spidery growth is formed through which the medium can easily be seen, and no close mat is produced. In places, however, the hyphae become aggregated into small lumps, which appear as whitish or fawn spots. On a medium containing 1 % of peptone and 2 % of glucose, a much more vigorous growth develops, and a very thick mat is formed, consisting of smooth, rounded, mammiform lumps. These are mainly *ochraceous buff* and very tough in consistency. Sometimes a pore surface develops on them, and later this may become overgrown.

The aerial hyphae in a mature culture are mostly hyaline and empty of contents. They measure 3–5 μ across, being occasionally up to 6 μ wide. The hyphae have rather numerous septa and branch rather frequently, often at right angles to the main hyphae. A few oval chlamydospores (about 10 μ diam.) occur, some of these have pale brown contents. Clamp connexions have not been observed.

Physiological data. F. ulmarius appears to be unable to make vigorous growth on ordinary agar media. A fairly high concentration (up to 3 %) of peptone in the solution stimulates growth in a glucose peptone agar. The fungus grows rather slowly on malt agar, as the following figures show:

Temp. °C.	10	20	22	24	28	30	34	38
Av. daily increment, mm.	0·8	3·5	4·9	5·1	5·5	5·3	3·6	0

From this test it appears that the optimum temperature for growth is about 28° C. Hemmi (1932)

found that the fungus from *Cryptomeria japonica* grew most rapidly at 36° C. and made some growth at 44° C. This suggests that it was a different plant to the species on elm in this country.

Economic importance. ' *F. ulmarius* is the principal cause of butt rot of elm in this country, and is particularly prevalent in parkland and hedgerow trees, where the bark has been damaged. Bark wounds caused by deer are frequently infected by this fungus, and in one well-known deer park every elm examined was infected. Once a tree is infected nothing can be done to save it, and the tree should be felled in order to salvage as much of the timber as possible and to avoid the risk of the tree falling unexpectedly at a later date. There is no evidence that the fungus can continue to spread after the tree is felled, and the fungus has never been reported as causing damage in structural timbers.

Ganoderma applanatum (Pers.) Pat.

Syn. *Fomes applanatus* (Pers.) Gill. *Placodes applanatus* (Pers.) Quel.

Occurrence. *Ganoderma applanatum* in various forms is cosmopolitan and extremely common in most localities. According to Lloyd (1915) *Fomes leucophaeus* is a form with a hard pale crust, while *F. fasciatus, F. australis, F. oroflavus, F. nigro-laccatus* are tropical forms. *F. reniformis* is stated to be exactly similar but annual. Rea (1922) lists *laccatum* as a variety having the orifice of the pores yellow and *vegetum* as a variety with a white mycelial layer interposed between each stratum of the tubes. The various tropical forms of *Ganoderma applanatum* and the closely related species *G. lobatum* have been described by Humphrey & Leus (1931), who also list their hitherto recorded hosts, while a full description of the forms which occur in Japan is given by Yamano (1931) who recognizes *G. vegetum* as a separate species. The fungus is a wound parasite and causes an active heart rot in a wide range of host species: in this country it is most common on beech but has been recorded on most of the native dicotyledonous trees.

Sporophore. The fruit bodies appear on living trunks, usually somewhere near the base, and at a point where there has been a branch or other wound in the trunk, but they frequently continue to grow on the stumps of felled trees (see Pl. 3, fig. *b*). The sporophore is a broad flattened bracket of the typical *Fomes* type, which may sometimes be as much as 40 cm. across. The fruit bodies are often imbricated and their upper surfaces may be flat or zoned and raised in lumps. The colour of the upper surface is usually a rich rusty brown, varying in tone from *russet* to *mars brown*, and is usually dusted with a thick deposit of ferruginous spores. The texture of the sporophore is very firm, and old specimens become hard and extremely tough, but the upper surface can easily be indented with a finger nail when fresh. The thick flesh is a rich brown, and on being torn has a silky fibrous appearance. The tubes, 1–4 cm. long, are brown, but the orifices of the minute pores are white so that the lower surface appears whitish or yellowish until it is scratched. The margin of specimens in active growth is white. The ferruginous spores are broadly elliptical and minutely echinulate. They measure $8–12 \times 5–8\,\mu$ (Bourdot & Galzin). They have been described as truncate at the base, but White (1920) pointed out that it is the apex which is broadly conical and which on drying appears truncated. A continuous heavy discharge of spores is maintained over a long period.

The rot. The appearance of the decay caused by this fungus has been well described and figured by White (1920). The first stage of decay is a whitish mottling, the whitish areas generally running across the grain of the wood, so that the latter eventually tends to break up into rectangular pieces. Only in the very late stage of the rot is the decayed wood a uniform white, by which time it is very soft, light in weight and spongy. In living wood which has been attacked by *G. applanatum* there is always a narrow band about 0·5 cm. wide of dark brownish discoloration marking the extreme limit of advance of the fungus (Pl. 6, fig. *a*). This band is different to the so-called 'black' line which, according to White (1920), occurs in wood decayed by *G. applanatum* only when another fungus is present. Under the microscope these dark bands are seen to consist of a layer of cells in which tyloses are abundant and 'wound gum' is present in varying amounts. As decay progresses these dark lines move forward into sound wood gradually developing on the outside and disappearing on the side next the decayed area.

Microscopic details of the rot. Mycelium is fairly plentiful in the decayed wood and consists of rather fine hyphae, $0·5–3·5\,\mu$ diam., which bear clamp connexions and penetrate the walls freely, making bores which are rather narrow at first but later become enlarged. In an advancing area of decay the penetrating hyphae tend to run parallel across the grain.

Fungus in culture. On malt agar growth starts from the transplant with fine, appressed, radiating hyphae which tend to aggregate into fine silky tufts; they do not always radiate out in straight lines,

but tend to curve out in the same direction. The mat quickly becomes opaque and white, has at first a smooth chalky surface, and as it enlarges a zonation may be produced by the formation of zones of short 'stubbly' growth. Drops of secreted liquid are often produced, beading the mat at the lower end. The mat becomes extremely tough and, only when very old, hard and rather brittle. The mat is at first white and later develops various pale yellow and light brown tints, different isolations exhibiting varying depths of colour. Among tints observed are *marguerite yellow*, *pinkish buff*, and *light pinkish cinnamon*. Fritz (1923), who described the culture of this fungus very fully, also noted *ecru olive*, *citrine drab* and *deep greyish olive*. Occasionally small sporophores are formed in culture on agar (see Pl. 2, fig. *e*). Hopp (1938) described the appearance of typical sporophores in cultures on sterilized poplar wood (see below). The young advancing hyphae are thin-walled, hyaline, mostly about 4 μ in width and bear a few clamp connexions. The aerial hyphae in the mature mat are mainly colourless, thick-walled and are rather narrow (1–4 μ) and without clamp connexions. In places the hyphae may swell irregularly and anastomose, giving a closely interwoven mat. On the submerged hyphae ovoid accretions of crystalline material occur, and the older hyphae may become completely incrusted with crystals which also occur plentifully in the surrounding medium. The submerged hyphae are frequently much inflated (up to 10 μ diam.), but no definite chlamydospores have been seen.

Physiological data. The rate of growth in culture is moderate, the average daily increment in diameter at the optimum temperature which is just below 30° C. being about 10 mm. The maximum temperature for growth is about 38° C. and only very slow growth occurs at 10° C. The fungus brings about a white rot attacking the lignin as well as the cellulose and pentosans (W. G. Campbell, 1932). Wood in an advanced stage of decay gives only a faint reaction with phloroglucin. White (1920) found that the germination of the spores was erratic and that they lost their viability after about 6 months. Germination, when it did occur, took place after about 48 hr. in water or nutritive medium, the germ tube always emerging from the truncated apex of the spore. Hopp (1938), who succeeded in obtaining typical sporophores on sterilized poplar wood when the cultures were aerated at 75% relative humidity, summarizes the environmental conditions necessary for their formation as follows: (*a*) exposure of the surface mycelium to air of normal oxygen concentration, (*b*) sufficient moisture supply to the mycelium within the substratum, (*c*) continuous but moderate desiccation of the surface mycelium by exposure of the wood block to moist but not saturated air.

Economic importance. *G. applanatum* is probably the most important cause of heart rot in beech in this country and causes a great deal of damage to large, over-mature, parkland trees. Since it usually gains entrance through branch wounds it is mainly large trees with big boughs liable to be damaged, which succumb. It occurs on many other leafy trees, causing a similar heart rot, but it is important principally as a parasite of the beech. Much of the damage caused by this fungus used to be ascribed to *Fomes fomentarius*, with which it was confused, probably because the latter fungus is common on the beech on the Continent.

Pleurotus ostreatus (Jacq.) Fr.—oyster fungus

Occurrence. *Pleurotus ostreatus* is widely distributed in Europe and America, and has been reported from Africa, Australia and India. In Great Britain it is common on a wide range of broad-leaved hosts, occurring most often on beech, and is occasionally found on softwoods. The fruit bodies usually appear on the trunk at or near a wound or branch stub.

Sporophore. The thick, fleshy, fruit body is fan-shaped and measures 7–13 cm. across. It may be completely sessile or extended behind into a short oblique stem-like base. The under-surface is covered with whitish, decurrent gills which anastomose near the base. The upper surface, smooth and moist in young specimens, is at first deep bluish grey and later becomes pale grey or fawn. The white flesh is soft when young but becomes firm on drying. The spores are colourless under the microscope, but may show a lilac tinge in the mass. They measure 9–11 × 4·5–6 μ (Rea). The fungus is very variable and a large number of forms has been described. Fruit bodies developed in the dark may show a greatly developed stem and little or no pileus.

Gross characters of the rot. In hardwoods the fungus causes a white flaky rot, the decayed portions usually being surrounded by a narrow dark brown invasion zone. In a rather unusual instance of decay in a pitch-pine post, the fungus was found to have caused complete separation of all the annual rings.

Microscopic details of the rot. Hyphae are plentiful in decayed beech wood and sometimes are massed in the vessels, building up strands formed by a number of hyphae running parallel to each

other. Most of the hyphae in the wood are 1–2 μ diam., but hyphae up to 6 μ across occur in the vessels. They bear numerous clamp connexions and penetrate the cell walls causing small cylindrical bore-holes 1–2 μ wide. The microscopic changes brought about in maple wood were described and illustrated by Learn (1912), who noted that the hyphae enlarge the pits after passing through them and that the lumina gradually enlarge; the attack appears to proceed outwards so that the tertiary wall is attacked first and the middle lamella is broken down only in the very last stage. Medullary rays seem to persist longer than other elements in the wood. Microchemical tests indicated that delignification took place more rapidly in the spring wood than in the summer wood.

Fungus in culture. On malt agar the fungus produces a dense, white, woolly felted mat with the aerial mycelium spreading round the sides of the culture tube; finally, the mat becomes tough and almost leathery and may show patches of cream colour. Usually small fruit bodies with elongated stipes and small pilei are formed—sometimes the latter fail to develop and only the stipes are formed—these may branch and form a coral-like growth. Grown in the dark on bread, the mycelium is at first white; after 3 weeks the centre becomes flesh ochre. On the same medium in the light drops of yellowish red liquid are exuded. The colours produced in cultures on bread serve to distinguish *P. ostreatus* from the other common species of *Pleurotus*, the cultures of which remain white on this medium. The hyphae in an agar culture which vary from 3 to 7 μ diam., bear numerous clamp connexions, and occasionally crystalline accretions. Crystals are formed plentifully in the medium.

Physiological data. The fungus grows rapidly on malt agar, the average daily increment in diameter of a colony being 17·2 mm. at the optimum temperature which is 27° C. It is also capable of making comparatively rapid growth at low temperatures, spreading 5 mm. per day at 10° C. W. G. Campbell (1932), who analysed sawdust decomposed by the fungus, found that it attacked all the constituents of the wood, causing a typical white rot. A blue reaction is given when guaiacum solution is added to a medium in which the fungus has been growing, indicating the production of oxidizing enzymes. Learn (1912) found that the spores germinate readily in 3–4 days in water and nutrient solutions, and that they retain their viability for at least 4 months.

Economic importance. *P. ostreatus* causes a considerable amount of damage to beech and other broad-leaves trees and can bring about rapid decay once it has gained entry. It has been reported as a cause of decay in almond trees in California and of figs in north Africa. The fruit body is edible, and in Japan it is said to have been cultivated for food.

Polyporus betulinus (Bull.) Fr.—birch polypore

Syn. *Ungulina betulina* (Bull.) Pat.

Occurrence. *Polyporus betulinus* is widely distributed and common in Europe, Asia and America, occurring wherever birches are grown. It has been reported from Australia, but Lloyd (1915) states that in that country it appears to be represented by the closely related *P. eucalyptorum*. It is very rarely found on any host other than species of birch. Macdonald (1937), in a comprehensive study of the species, suggests that it has become increasingly common in this country. The fruit bodies occur on dead or, less commonly, on living branches and trunks and continue to develop on the timber for several years after it has fallen.

Sporophore. The fruit body of *P. betulinus* is characteristic and always easily recognizable. It varies in breadth from 5 to 30 cm., but is usually 12–15 cm. across, reaching its full size in about 6 weeks after the first appearance of the fruit body initial as a firm round knob. It is always rounded, being hoof-shaped to kidney-shaped, with an obtuse edge. It is attached by a narrow base which sometimes forms a thick short stalk. The upper surface is frequently humped; when young it is smooth, though the skin on the upper surface may crack as it ages. The top is a uniform pale brown, but the margin is usually lighter while the pore surface is white. The tubes are 2–8 mm. long, with small mouths (3–4 to the mm.). The colourless cylindrical spores measure 4·4–5·5 × 1·4–2 μ (Macdonald). Conditions influencing the discharge of the spores and their cytology were studied by Macdonald (1937), who showed that the fungus is heterothallic, the spores being of two kinds.

Gross characters of the rot. In an advanced stage of decay the wood is reddish brown and breaks up into square-edged blocks, usually separated by thin sheets of whitish mycelium. Eventually the rotted wood falls into powder. The fungus attacks both heart and sapwood equally. In blocks of wood artificially infected and rotted by the fungus Macdonald observed dark-coloured zone lines.

Microscopic details of the rot. The spread of the mycelium and its effect on the elements of the wood were first described and figured by Mayr (1884). Hyphae are plentiful in decayed wood and bore freely through the walls, enlarging the bore-holes after penetration; they bear clamp connexions, occasionally of the 'medallion' type.

Fungus in culture. The appearance of the fungus in culture was fully described by Macdonald (1937). The mat, at first thin, soon becomes cotton-woolly, and may fill the bottom end of a tube culture with a dense plug. The colour, at first white, later becomes faintly pink or pale brown (*pinkish buff* to *tilleul buff*). A flat pore surface or rounded sporophore may develop at the upper end of the slant. Dark lines similar to those formed in wood blocks on which the fungus is cultivated may be produced in agar cultures, and associated with these may be small sclerotial bodies. The mature culture has a sweet, spicy smell, somewhat like that of roast apples. It consists mainly of fibrous, thick-walled hyphae, with few septa and no clamp connexions. The young advancing hyphae vary from 1 to 7 μ diam. and bear a few simple clamp connexions. Macdonald did not note the presence of chlamydospores, but the authors have observed them on the submerged mycelium of cultures grown in the dark, and occasionally in cultures grown in the light. They may be round (10–28 μ across) or oval (10–26 × 8–12 μ). Numerous crystals are formed in the media.

Physiological data. The rate of growth on agar is moderately slow, being about 7 mm. per day at the optimum temperature for growth which is about 26° C. Hemmi & Kurata (1931) found that their strain grew at somewhat higher temperatures with an optimum about 28° C. and a maximum just over 36° C. The rates of growth on 2% malt agar at various temperatures are as follows:

Temp. °C.	10	17	22	24	28	30	34
Av. daily increment, mm.	2	3·1	5·8	6·7	6·3	3·0	0

Macdonald (1937) examined the enzymes of the fungus and concluded that it produces enzymes capable of attacking lignin. Though the rot appears to be a brown one, he found some evidence of oxidase activity, as a culture produces a dark ring around itself on a medium containing gallic acid. Hemmi & Kurata (1931), on the other hand, classified the fungus as belonging to the cellulose-destroying group, and LaFuze (1937), although unable to detect oxidases, found abundant reductases. Probably its attack is mainly confined to the cellulose and the associated carbohydrates.

In the laboratory *P. betulinus* will grow on and decay various species of wood, such as beech and sycamore, although it is more or less confined to birch in nature.

Economic importance. In England *P. betulinus* is by far the most important cause of heart rot in the birch; in Scotland it is also the most common fungus on this tree, but in parts of the Highlands *Fomes fomentarius* is equally prevalent. *Polyporus betulinus* causes a great deal of damage to trees which have been injured or weakened. In woods near industrial areas the trees are sometimes heavily infected with this fungus. It does not require large branch wounds exposing mature wood in order to gain entry, as sometimes quite young trees showing no large wounds may be found to be infected. Control of the parasite is primarily a matter of good sylviculture, since the disease seldom appears to be serious on well-grown trees unless these are over-mature. Where it is common, infected trees should be cut down and the wood burnt in order to prevent the further development of fruit bodies on the felled timber.

Polyporus cuticularis (Bull.) Fr.

Syn. *Xanthochrous cuticularis* (Bull.) Pat.

Occurrence. *Polyporus cuticularis* is moderately common in this country and on the Continent, while in America it is stated to occur in the greatest abundance. Here it usually grows on beech or hornbeam; it has also been reported on maple, oak and sweet chestnut.

Sporophore. The fruit bodies are bracket-shaped and usually imbricated (see Pl. 3, fig. *a*). They vary from 7 to 30 cm. across. The upper surface, which is very hairy and zoned when young, is rusty brown. The flesh, which is rather thin, is hard and fibrous and dark brown. The fine, concolorous tubes are 3–10 mm. long. The pore surface, which has a whitish, glistening appearance when young, later becomes dark brown. The elliptical spores are brownish yellow and measure 6–7 × 4–5 μ (Rea). Brown spinules (setae), which are often branched, occur rather irregularly or may be absent. Sometimes fruit bodies of this species are not easy to distinguish from those of *P. radiatus*, but they can generally be recognized by their larger size, more shaggy upper surface, by the absence of the bright, golden yellow margin, and by the somewhat larger, more deeply coloured spores.

Gross characters of the rot. The fungus causes a white, stringy rot, in which chocolate-coloured zone lines are usually formed, particularly near an exposed surface.

Microscopic details of the rot. Hyphae are plentiful in decayed wood; they are of two types, (*a*) fine, thin-walled, hyaline hyphae 0·5–3 μ across, and, (*b*) much-branched, rather knotted, brown hyphae 2·5–6 μ across, which tend to occur mostly in the rays, which are usually severely attacked. The dark hyphae are occasionally much swollen, and frequently septate. Numerous branch hyphae are given off at right angles from the main hyphae, and penetrate the cell walls. They usually swell out on contact with the wall and after passing through it. At first the bore-holes are rather fine; later they enlarge, and may coalesce to form an irregular shaped hole. No clamp connexions have been observed.

Fungus in culture. On 2 % malt agar, in the light, it first forms a sodden, colourless mat, which begins to develop traces of yellow around the inoculum; in the dark a more woolly mat develops, which is denser than that of *P. radiatus*. Finally, a thick, woolly mat is formed, which is inclined to be silky, with a tendency of the hyphae to radiate outwards from the inoculum (see Pl. 2, fig. *i*). At first the mat is colourless, but it soon becomes *pale ochraceous yellow*, then *raw sienna* to *antique brown*. In the dark, on malt containing 1 % malic acid, the tints noted were *baryta yellow*, *empire yellow*, *xanthine orange* and *mars yellow*. No growth took place on 5 % malt agar and 1 % malic acid *in the light*, which helps to distinguish it from *P. radiatus*, which is less sensitive to light and grows on acid malt in the light. The hyphae average about 3 μ wide, i.e. somewhat larger than those of *P. radiatus*. They are sometimes aggregated together to form loose strands, apparently due to the rather spiral growth of the hyphae, which leads to a rope-like structure. Some thick-walled, coloured hyphae and long, pointed, coloured setae (up to 80 μ long) may be found. No clamp connexions or secondary spores have been observed.

Physiological data. The fungus grows at a moderate rate in culture. The average daily increments in diameter of cultures on prune agar at different temperatures were as follows. Prune agar was used instead of the usual malt, as the fungus makes more regular and vigorous growth on it.

Temp. °C.	10	20	22	24	28	30	34	38
Av. daily increment, mm.	1·7	3·4	4·4	5·9	9·5	9·9	9·5	trace

Thus the fungus appears to be sensitive to temperature changes and to have its optimum temperature for growth at about 30° C.; above 34° C. the rate of growth falls off very rapidly, the maximum lying at about 38° C.

It does not appear to produce any appreciable amount of acid when grown in liquid media, and the *p*H of a culture solution in which it had grown for 2–6 weeks had not changed appreciably. This is unusual, for most white-rot fungi cause at first an increase in acidity of the medium, although subsequently the *p*H may return to its former figure. The fungus appears to be sensitive to acid conditions, particularly in the presence of light, and no growth took place in the light on 5 % malt agar acidified with malic acid.

Economic importance. The fungus is not sufficiently common to be of major importance in this country. Pilat (1926) stated that it is occasionally found in central Europe, causing rot of deciduous trees. Here it is more frequently found on fallen logs and stumps than on living trees. Bourdot & Galzin (1927) stated that it seldom kills a tree, but may cause the formation of an excrescence which is known as *chandeau*.

Polyporus giganteus (Pers.) Fr.

Occurrence. *Polyporus giganteus* is widely distributed throughout Europe and has been reported from North America. In England it is fairly common on beech and is recorded as occurring on a number of other broad-leaved trees.

Sporophore. The fruit body consists of a rounded mass of imbricated pilei attached to a central boss, and it may attain a large size. It develops either on stumps or on decayed roots at a distance of some feet from the base of the trunk. The individual pilei are brownish yellow to chestnut brown on top, with a yellowish margin. The upper surface is somewhat granular and fibrillose. The lower surface, which is covered with small rounded pores, is creamy white, but darkens on being touched. The tubes are 4–6 mm. long, and the colourless, rather oily-looking subglobose or broadly elliptical spores average 6 × 5 μ.

The rot. The fungus causes an active white rot which is mainly confined to the roots and base of the trunk. The hyphae, which are abundant in decayed wood, are mostly 2·5–4 μ across, but

occasionally hyphae 6–8 μ wide may be found, and some of them are very uneven in width, with swellings. They pass mainly through the pits, and when they do penetrate the cell walls, make only fine bore holes. The hyphae in the wood are mainly hyaline and have not been observed to bear clamp connexions.

Fungus in culture. The culture is rather characterless, the mat consisting of soft, white, cotton-woolly growth, which tends to grow up the sides of the tube and later to become somewhat tougher and more leathery. Finally, tawny or cinnamon brown patches appear at the upper end of the slope. The hyphae are colourless, and average 2 μ diam. Some large hyphae 7 μ across, with numerous uneven swellings, may be observed, and in old cultures terminal chlamydospores up to 25 μ across may be found.

Physiological data. Rate of growth in culture is moderate, a 10 × 3·5 cm. slope being covered in 18 days.

Economic importance. No experimental work on the parasitism of the fungus is known. Pilat (1934) states that it infects only sickly old trees. Probably it is usually of secondary importance, but its action on the roots may lead to the overthrow of the tree.

Polyporus hispidus (Bull.) Fr.

Syn. *Xanthochrous hispidus* (Bull.) Pat.

Occurrence. Polyporus hispidus is common and widely distributed in Europe, and has been reported from America, India and Australia. In this country it most frequently attacks the ash (*Fraxinus excelsior*) but is also commonly found on apple, and occasionally on walnut, elm and plane. In America it is stated by Baxter (1925) to attack only the black ash (*F. nigra*) and never the white ash (*F. excelsior*).

Sporophore. The annual fruit body is shaped either like a hoof or a short, thick, broad bracket 10–30 cm. across with a shaggy upper surface and thick, spongy, fibrous flesh (see Pl. 4, fig. *d*). At first it is a rusty reddish yellow, when mature an iron rust colour, and finally when old and dead it becomes practically black. The tubes are 2–3 cm. long and the reddish yellow pores, 0·15–0·3 mm. across, are at first round, and then become torn. The subglobose yellowish spores measure 9–10 to 7–8 μ. Sometimes yellowish brown cystidia are present. The fruit bodies which develop on the tree while it is still alive usually appear at the point of infection.

Gross characters of the rot. Infection occurs at a branch stub and the rot spreads up and down the tree. In the first stage of attack there are always whitish or yellowish 'flames' of discoloration which are limited by a brown zone resulting from the formation of a gummy material. The development of this gum is particularly plentiful in plane trees, where the disease always takes the form of a gummy rot. In walnut the wood in the final stage of the rot is converted into a spongy yellow mass. In the case of ash the decayed wood is definitely lighter in colour and softer than sound wood and cracks along the annual rings when dried.

Microscopic details of rot. The action of the fungus on the cell walls and the microscopic appearance of the hyphae in the wood were described and figured in detail by Nutman (1929) from whose paper the following account is taken. At first the hyphae develop mainly in the medullary rays and parenchyma, and to a certain extent in the vessels; later the wood fibres are attacked; the hyphae average 1·5–2 μ diam. The hyphae bore freely, enlarging the bore-holes after passage, up to 3–5 times their own width. Nutman observed that in blocks which had been exposed to the fungus for 4 months the penetration was almost entirely by means of the pits, but that in blocks in which decay had progressed further penetration through the cell walls could be seen frequently and was of two types, (*a*) by means of the tip of a young hypha, (*b*) by means of a peg sent out from an older, thicker hypha. When the fungus is in active growth the hyphae are colourless, but in an advanced stage of decay thick-walled, brown hyphae occur, and these form a typical zone line. No clamp connexions have been observed.

Fungus in culture. Polyporus hispidus grows readily but slowly on most ordinary culture media. On 2% malt agar it forms a thick, silky to hairy, plush-like mat, broadly zoned, uniformly coloured—*primuline yellow* to *mustard yellow*, becoming *antique brown* or *buckthorn brown*. In common with most fungi which form a richly coloured mat, it develops more vigorously in the dark. Normally it does not fruit in culture, but in one culture on turnip extract Nutman observed a small fruit body. The young colourless hyphae on agar measure 3–5 μ across, while older yellowish hyphae average 5 μ across. No clamp connexions and no secondary spores have been observed in cultures.

Physiological data. The fungus grows somewhat slowly on malt agar, the optimum temperature being about 30° C. The following are the rates of growth at various temperatures:

Temp. °C.	10	16	20	22	24	28	30	34	38
Av. daily increment, mm.	0·9	1·8	2·7	3·7	4·1	5·7	6·8	5·3	0

Nutman (1929) measured the rate of spread of the fungus in blocks of wood and found it to be 5 mm. per month across the growth rings and at least 7 mm. per month parallel to the grain. He found that enzymes capable of attacking the lignin were produced in addition to those which cause hydrolysis of the carbohydrates. He considered that oxidases were present but the test described, which involved the addition of hydrogen peroxide, is usually considered to demonstrate the presence of a *peroxidase*. Since it was later shown by W. G. Campbell (1931) that the rot is of the white-rot type in which both lignin and cellulose are attacked, it is probable that oxidizing enzymes capable of breaking down the lignin are present. Lutz (1939) showed that *P. hispidus* can produce a 'mutase' which breaks down aldehydes into alcohol.

Mechanical tests by Cartwright *et al.* (1936) on small samples exposed to infection by this fungus proved that the toughness (resistance to a suddenly applied stress) is quickly affected, being reduced by 27% after 2 weeks' exposure, and by about 90% in 12 weeks, at which stage the test pieces were extremely brittle, though outwardly but little altered in appearance and having lost only about 10% of their weight through fungal decay. It is probable that the 'brashness' (liability to snap with a brittle, short fracture) of some samples of apparently sound ash is due to incipient decay in the growing tree caused by this fungus. The bending strength of test pieces infected with *P. hispidus* decreased at a much slower rate than the toughness, only 14·3% reduction being noted after 12 weeks.

Economic importance. *P. hispidus* is probably the most important cause of decay in standing ash trees and causes much damage, especially to hedgerow and isolated trees which are liable to have their branches broken off by the wind. The fungus can attack living cells and become truly parasitic. Since ash timber is used largely for purposes where high strength and great toughness are required, such as in sports goods, any reduction of strength is serious. Even very slight decay by this fungus greatly reduces the toughness of ash, and any timber showing the slightest traces of decay must be rejected. This sometimes involves cutting away for some distance longitudinally beyond the last visible signs of rot. Prevention of attack by this fungus depends, as in the case of most trunk rots, on the avoidance of wounds.

Polyporus radiatus (Sow.) Fr.

Syn. *Xanthochrous radiatus* (Sow.) Pat. *Polyporus alneus* Persoon (according to Lloyd)

Occurrence. *Polyporus radiatus* is common on the alder in Europe and North America. It is occasionally found on birch, willow and poplar, while the form which occurs on beech has been described as a separate species, *P. nodulosus* Fr.

Sporophore. The fruit body is a thick, short bracket, 2–6 cm. across, usually dimidiate and imbricate. The upper surface is yellowish to rusty brown, darkening as it ages, while the margin remains golden yellow. It is velvety and shows raised radial lines. The flesh is hard, fibrous, and yellowish brown; the fine tubes, which vary from 0·5 to 1·0 cm. long, are the same colour. The small pores are rusty brown, but the under-surface has a silvery and glistening 'shot' appearance when fresh. The elliptical spores, which average $5 \times 4 \mu$ (Rea), are colourless or pale yellow. Dark brown cystidia are usually abundant.

Gross characters of the rot. Alder wood rotted by this fungus is whitish or pale biscuit coloured, light in weight, and breaks with a flaky fracture. Narrow, dark brown zone lines may be formed just below the broken surface of infected wood which is drying out.

Microscopical details of the rot. Narrow hyphae about 1μ across are fairly plentiful in decayed wood, and slightly wider, coloured ones up to $2{\cdot}5 \mu$ across, with moderately thick walls, also occur. No clamp connexions or chlamydospores have been observed. The hyphae penetrate the cell walls by means of very fine bore-holes; they also cause enlargement of the pits, and erode longitudinal channels in the walls. The zone lines are formed by a narrow zone of pseudoparenchyma in the vessels, built up of much-branched and frequently septate brown hyphae. A brownish gum-like material is also present in decayed wood.

Fungus in culture. On malt agar, growth begins with a thin appressed mycelium; in the light there is at first very little aerial development, but in the dark a loose, cobwebby mycelium soon

develops. Later a velvety, slightly tufted, compact mat is formed, which eventually has a somewhat powdery appearance. The culture exhibits various yellow brown tints, which are more pronounced in cultures grown in the dark. The following were noted in cultures: *light orange yellow*, *antimony yellow*, *raw sienna*, *antique brown*, *sudan brown*. In an older culture the lower end of the slope usually shows the paler tints. The submerged mycelium is a rich, dark brown (*chestnut brown* or *bay*), giving the medium below the mat a similar colour. This feature is not present in cultures of *Polyporus cuticularis*, which otherwise closely resemble those of *P. radiatus*.

Physiological data. The fungus grows very slowly on malt agar; the average daily increments in diameter of cultures at various temperatures were as follows:

Temp. °C.	10	20	22	24	28	30	34	38
Av. daily increment, mm.	1·4	1·7	2·0	2·8	2·4	2·1	0·7	Nil

The optimum temperature for growth thus appears to be just above 24° C.

Economic importance. *P. radiatus* is by far the most important cause of heart rot of alder in this country and is responsible for a great deal of decay in suppressed and damaged trees. There is little information available as to whether the fungus can cause the death of living cells and become actively parasitic in vigorous trees.

Polyporus squamosus (Huds.) Fr.—saddle-back fungus

Syn. *Melanopus squamosus* (Huds.) Pat. *Cerioporus squamosus* (Huds.) Quél.
Polyporellus squamosus (Huds.) Karst.

Occurrence. *Polyporus squamosus* is widely distributed, being common both in this country and in Europe on a number of broad-leaved trees. It has been reported from India, Australia and from America where it is described as rare. In Great Britain it is most common on elm and sycamore, but is frequently found on other species of *Acer*, also on *Pyrus* spp., on walnut, and occasionally on other broad-leaved trees. The fruit bodies usually occur at a branch scar often fairly high up on the trunk or on branches. The synonymy and varietal types were described in detail by Graff (1936) and Pilat (1936), the latter giving some excellent illustrations.

Sporophore. The fan-shaped fruit bodies grow very rapidly, often taking only about 14 days to reach a diameter of 25 cm. Usually there is a short thick lateral stalk which is dark-coloured at the base. The fruit bodies vary greatly in size, from the small specimens a few centimetres across which develop on small branches, to brackets as much as 60 cm. across. At first they are soft and fleshy and later become leathery. They are annual, soon decaying and becoming infested with maggots. The upper surface which is pale fawn has numerous dark brown, appressed scales which are characteristic of the species. The pore surface is a pale cream colour and the pore mouths when mature are large, angular and torn. The colourless oblong spores which measure $10–12 \times 4–5\,\mu$ (Rea) are produced in immense numbers. The morphology and anatomy of the sporophore were described in great detail by Oehm (1933).

The rot. Infection takes place at a branch wound and the fungus, having gained an entrance, penetrates to the centre of the trunk and then travels up and down decaying the heartwood from within outwards, which eventually results in a hollowing out of the trunk (see Pl. 5, fig. *a*). It brings about a spongy or stringy white rot in which there is usually a considerable quantity of white mycelium. Sometimes a tough brown stromatic sheet may be found lining part of the cavity in a hollow trunk. On cutting into the decayed wood, particularly near the place where a sporophore is attached, blackish 'zone lines' are usually visible; the development and anatomy of these plates have been described in detail by Campbell & Munson (1936) who consider them as the limiting layers of a 'pseudosclerotium' embedded in the wood.

Microscopic details of the rot. The hyphae in the wood may be narrow and thick-walled or in the vessels wide and thin-walled with diam. up to $7–11\,\mu$, some of the latter showing oval or elongated swellings. They bear clamp connexions and penetrate the cell walls freely, enlarging the bore-holes and pits after penetration. A progressive thinning of the cell walls is brought about, the less heavily lignified tissues being attacked first. The mycelium in the wood tends locally to form small white irregular strands or sheets which break up the wood into irregular pieces. Campbell & Munson (1936) described and illustrated the pseudoparenchyma of dark brown bladder-shaped cells which constitutes the blackish plates bordering the pseudosclerotium.

Fungus in culture. The appearance of the fungus in culture on malt agar was described by Campbell & Munson (1936). The fungus grows slowly, forming at first a loose white cottony mycelium which soon tends to aggregate at the sides of the tube forming groups of short papillae. Soon, a brown skin is formed over most of the slope—this is at first *olive brown* or *buff brown* and finally becomes *snuff brown*. It has a characteristically dusty appearance due to the presence of large numbers of oidia. Occasionally small fertile sporophores are formed in culture (see Pl. 2, fig. *g*) but more frequently only the stipes develop; these may be lobed and branched, giving rise to antler-like outgrowths. The hyphae are mostly fine, the first formed being 2–3 μ across, while in the mature mat they are mostly about 1 μ with a few up to 3 μ; they are closely matted and mostly thick-walled. The hyphae submerged in the medium average 2–2·5 μ diam. and bear numerous simple clamp connexions. Numerous oidia are formed which are irregular in shape and size but are usually ovoid and average 18 × 8 μ. Numerous rod-shaped crystals, sometimes aggregated into clusters, are formed in the medium.

Physiological data. The germination of the spores, which takes place readily in nutritive solutions, was described by Buller (1906), who also investigated the enzymes produced by the fungus. The rates of growth on agar at various temperatures were as follows:

Temp. °C.	10	17	22	24	28	30	34	38
Av. daily increment, mm.	2·1	4·7	6·3	7·9	5·0	1·8	0·3	0

The optimum temperature for growth thus appears to be about 24° C., which is somewhat lower than the average.

Economic importance. Polyporus squamosus is the principal cause of heart rot in elms in this country and is responsible for the destruction of large numbers of hedgerow and parkland trees which are overthrown by the wind after having become hollowed out as a result of attack. Elms are notoriously liable to heart rot and sometimes collapse without warning in calm weather. Prevention of attack by *P. squamosus* is best achieved by cutting off close to the trunk any snags left after branches have fallen. The cut should be made vertically as close to the trunk as possible, and the cut surface dressed immediately with paint or antiseptic. Overhanging and dangerous branches should be removed before they break off, but pollarding of elms should always be avoided. It is very difficult to prevent decay penetrating into a large wound which exposes the heartwood, especially when the cut has been made horizontally. When quite young the fruit body is stated to be edible; when it is mature it has been used for making razor strops.

Polystictus versicolor (Linn.) Fr.

Syn. *Coriolus versicolor* (Linn.) Quél.

Occurrence. Polystictus versicolor is cosmopolitan, and very common in all temperate countries. It exhibits a wide range of forms, some of which are not easy to distinguish from the nearly related species *P. zonatus* and *P. velutinus*. It grows on many different kinds of hardwoods and can attack the sapwood of practically all timbers, while occasionally it may be found even on coniferous wood. Usually it is saprophytic and only occurs on felled timber or on the parts of trees which have already been killed by some other agency, but it has been described as causing a heart rot in *Catalpa speciosa*. It is found most frequently on timber in contact with the soil such as fence posts, logs lying in the forest, and old stumps, and so on. In timbers in which the heartwood is naturally durable, such as oak, its attack is mainly confined to the sapwood, but logs of less resistant species, such as beech, may be entirely destroyed by the fungus. It is often found in damp mines causing decay in the hardwood props.

Sporophore. The typical fruit bodies are thin, tough, imbricated brackets 3–8 cm. across, greyish or brownish on top with a cream pore surface beneath. The upper surface is always velvety, with concentric *satiny zones* of various colours. Fruit bodies developed in darkness as in a coal mine are pale creamy yellow or almost white, and less tough than the normal ones. The white tubes are very short and the pores which are at first small and round become torn and irregular as they develop. The oblong spores measure 6–8 × 3 μ (Rea) and appear cream in the mass, but colourless under the microscope.

Gross characters of the rot. The rot caused by *Polystictus versicolor* is typical of that caused by many saprophytic fungi in hardwoods. The first indication of decay in timbers such as ash and beech

is a white flecking; often boards of these timbers which have been stacked with insufficient ventilation show isolated spots of discoloration which penetrates only a short way into the surface of the wood. Each of these spots represents a separate focus of infection where a spore has germinated and set up a local area of 'dote' (incipient decay). As the rot progresses the wood becomes paler in colour and lighter in weight till in the last stage it is practically white and very light in weight. Scheffer (1936) suggested that this loss in colour is due to decomposition of the water-soluble colouring materials and is not the result of the destruction of lignin. Occasionally in blocks artificially rotted in the laboratory blackish specks are present on the surface of the decayed wood. *P. versicolor* can cause the complete destruction of wood (Findlay, 1940). Wood even in a very advanced stage of rot retains its original shape and volume and no shrinkage cracks appear. There is nothing very characteristic about the rot, but sometimes the identity of the fungus can be recognized in the absence of fruit bodies, by the presence on the surface of small sheets of whitish mycelium touched in places with the brownish tints typical of the sporophore.

Microscopic details of the rot. Mycelium is plentiful in decayed wood, and the larger vessels may be choked with a weft of it. The hyphae are colourless and mostly very fine, 0·5–4·0 μ diam., but occasionally wider hyphae up to 7 μ across are found in the vessels. They bear numerous clamp connexions which are hooped up so that they resemble eyelets. The hyphae pass through the pits and also in the later stages of attack bore freely through the cell walls, forming rather small bore-holes about 2·5 μ across. Scheffer (1936) noted that considerable thinning of the cell walls occurs, indicating their progressive consumption from the lumen outwards.

Fungus in culture. On 2% malt agar a fine colourless mycelium closely appressed to the medium is first formed. At this stage aerial hyphae develop at the growing edge forming a slightly raised margin of more fluffy growth, whilst over the centre part of the culture the mat is at first thin so that the medium shows through. Later a tough, flat, closely-felted mat is formed, and finally it has the appearance of kid or chamois leather, being white or slightly tinted, *marguerite yellow*, *cartridge buff* or *pale ochraceous buff*. Malt agar on which *P. versicolor* is growing always becomes bleached. The young hyphae are rather fine, mostly 2–3 μ across, the width being fairly uniform, and they bear numerous clamp connexions which are usually raised into definite loops. The mature aerial hyphae, which are usually thick-walled and rather fibrous, bear only few clamp connexions and are 3–4 μ diam., though odd hyphae up to 10 μ across may be found. A few chlamydospores occur and quantities of crystals are formed in the medium.

Physiological data. Jay (1934) found that the spores germinate readily in water and in ordinary nutrient solutions, and that they retain their vitality for at least 3 months. The fungus grows fairly rapidly in culture, spreading over malt agar at the rate of about 2 cm. per day, at the optimum temperature which is 29° C. The maximum temperature at which any growth can take place is about 38° C. The respiration of this fungus at various temperatures and oxygen pressures was measured by Scheffer & Livingston (1937), who found that growth was most rapid for combinations of 29·5° C. with oxygen pressures of from 15 to 745 mm., while production of CO_2 per 'mat unit' was most rapid for the combination of 33·5° C. with oxygen pressure of 745 mm. Growth occurred with relatively least loss of carbon when the oxygen pressure was near the minimal for growth, without much regard to temperature.

Polystictus versicolor is very active chemically, causing a white rot in which lignin as well as cellulose is vigorously attacked, and is able to decompose many heavily lignified tropical timbers. It is possible that this activity is associated with a strong oxidizing power of the enzymes which it produces. There is evidence of a vigorous production of oxidase, an intense blue colour being rapidly produced when a guaiacum solution is added to a liquid culture of the fungus. Lutz (1939) showed that *P. versicolor* can produce mutase and phosphatase, enzymes capable of bringing about alcoholic fermentation which occurs during growth of this fungus under anaerobic conditions. LaFuze (1937) showed that *P. versicolor* can grow in unusually high concentrations of glucosides including 4% of saponin. He found, on the other hand, that it is checked by low (0·55%) concentration of tannin, with which it reacts to form a deep brown, suggesting that it is the oxidation products of tannin which inhibit growth. This sensitiveness to tannin probably explains why oak heartwood is so resistant to this fungus. Generally speaking *P. versicolor* is comparatively resistant to most antiseptics, both to tar oil preservatives and to aqueous solutions of toxic salts.

A great deal of work has been done on the chemical aspects of the decay brought about by this fungus; W. G. Campbell (1930) found that the pentosans and the lignin are attacked at an early stage and compared the effect on beech wood to that produced by acid-alcohol solutions under certain conditions. Lutz (1930) studied the later stages of decay, and concluded that the fungus

attacked successively the lignin, the cellulose and the pectic substances of the middle lamella, and that beech wood decomposed by this fungus becomes transformed into a gum-like material mainly composed of xylan with traces of galactan, mannan and levulosan. This gum becomes hydrolysed into intermediate substances and eventually into the corresponding sugars which are partly absorbed by the fungus and partly split up into alcohol so that finally, as a result of oxidation, traces of acetone and of glycuronic acid may be found. Scheffer (1936) made an exhaustive investigation of the effect of *P. versicolor* on the strength of red gum sapwood, and correlated the loss in strength with the chemical changes brought about by the fungus. He considered that the initial reduction in strength was due to 'the removal or alteration of relatively small amounts of cell wall lignin and soluble carbohydrates which had served to cement the fundamental cellulose units together'. He found that the relative proportions of the chief wood components were not materially altered in the decayed wood. Based on the weight of the original sound wood, the lignin, the pentosans not in the cellulose, the cold-water soluble and the 1% alkali-soluble components were first attacked, while the Cross & Bevan cellulose, the pentosans in the cellulose, and the strictly hot-water-soluble portions were little affected until part of the wood substance had been consumed.

The fungus is resistant to desiccation and can revive after long periods of dryness in wood. It is fairly resistant to heat: Montgomery (1936*b*) found that the fungus in small blocks of wood was alive after 60 min. but dead after 90 min. exposure to 55° C.

Economic importance. *P. versicolor* probably causes more decay in felled hardwoods than any other fungus. It is a frequent cause of 'dote' in ash and beech planks stored under insufficiently ventilated conditions, and is the most common cause of decay in hardwood posts. It is occasionally found on exposed parts of buildings and has sometimes been isolated from decayed oak window-sills, but it has never been found causing 'dry rot' in structural timbers, for it requires an abundant supply of moisture.

Poria obliqua (Pers.) Bres.

Occurrence. *Poria obliqua* is a complex species which may include several different species. Here, consideration is only given to the variety which forms abortive sporophores on birch. This fungus, which is common on birch in certain localities in Europe and America, has variously been described as an abortive sterile form of *Polyporus nigricans* Fr. or of *Fomes igniarius* (L.) Gill., but its identity remained in doubt until Campbell & Davidson (1938) demonstrated that it is connected with a *Poria* fructification which Overholts determined as belonging to the *P. obliqua* group. Findlay (1939) isolated, from an abortive sporophore collected on birch near Aviemore in Scotland, a fungus which proved to be identical with the one described by Campbell & Davidson. It appears therefore that this form of the fungus which in Russia is called 'Tchaga', and is widely distributed in that country, in Sweden, and North America, also occurs in this country, but its prevalence requires further investigation. It is probable that the fungus has often been seen before, but has been regarded as merely an abortive form of some *Fomes*.

Sporophore. The fertile sporophore has not been found in this country. Campbell & Davidson (1938) describe the fructification as occurring only under the bark of dead trees where it forms a thin dark brown poroid layer. The sterile outgrowth which was collected in Scotland consisted of a coal-black mass about 30 cm. across. It was very much cracked and extremely hard and brittle, and in general appearance suggested a large clinker. The interior of the mass was a dark yellowish brown, only a thin external layer being carbonaceous.

Gross characters of the rot. The fungus causes a white rot in which the annual rings separate very readily from each other giving the wood a laminated appearance. Rather narrow, dark brownish black zones occur especially near the surface of the wood. In later stages of the rot the medullary rays are almost completely destroyed (see Pl. 4, fig. *g*) and the surrounding cells become delignified. A weft of mycelium replaces the destroyed rays.

Microscopic characters of the rot. Hyphae are plentiful throughout the elements of the wood. They are mainly fine and make small bore-holes. No clamp connexions have been observed. The middle lamella is destroyed in an early stage of attack causing the cells of the wood to separate. The zone lines are formed by masses of dark-coloured hyphae which are often swollen as in *Armillaria mellea*, and dark gum-like material is also formed. Behind these zones hyphae are particularly numerous, take up the stain strongly, and appear to be especially active (see Pl. 4, fig. *f*).

Fungus in culture. The appearance of the fungus in culture is distinctive and quite different to that of *Fomes igniarius*. Growth on agar starts with white, woolly mycelium which gradually becomes *honey yellow* at the centre of the mat. As the mat grows the colour spreads outwards, but for some

time a white margin is left. In the mature culture *Isabella colour*, *Dresden brown* and *yellow ochre* tints can be seen. After about 6 weeks, fructifications are usually formed consisting of small lumps bearing brownish pores. The basidiospores measure 8–10 × 4–5 μ and have a large central gutta. Dark brown, thick-walled, bulbous setae occur in the hymenium. The majority of the hyphae appear yellowish orange, average 4–5 μ diam. and their walls tend to be thickened. Numerous brown setae 40–80 μ long occur and some conidia-like bodies which may be formed from sterile basidia. No clamp connexions have been seen.

Physiological data. The fungus grows fairly rapidly in culture, the optimum temperature for its growth being about 28° C. The following are the rates of spread on 2% malt agar:

Temp. °C.	10	20	22	24	28	30	34	38
Av. daily increment, mm.	1·8	4·5	6·4	7·4	10·9	9·6	2·6	0

It is surprising that a fungus having such a northerly distribution should have such a high temperature range. The culture gives a positive test for oxidases.

Economic importance. In America the fungus is reported to be common in certain areas in New England on birches which had been damaged by ice or badly cankered, and to cause a well-defined heart rot which extends up and down the trunk and which may lead to the tree breaking off at the point where the fruit body appeared. The prevalence and economic importance of the fungus in this country are not known. It is stated that in Siberia an infusion from the sterile fruit bodies is sometimes used instead of tea.

Stereum purpureum (Pers.) Fr.

Syn. *S. vorticosum* Fr.

Occurrence. *Stereum purpureum* is cosmopolitan and grows on a wide range of non-durable timbers after felling. It is important, as a parasite, mainly on trees and shrubs belonging to the natural order Rosaceae, in which it causes the disease known as 'Silver Leaf'.

Sporophore. The fruit body consists of a thin, tough skin which may lie appressed to the substratum or be reflexed to form an irregular wavy-edged bracket, which is finally greyish and hairy on the upper surface. The hymenium is even and smooth, is at first a vivid lilac or purplish colour but, as the fruit body ages, it becomes purplish brown and finally pale fawn. The colourless, oblong or oboval spores measure 6–8 × 3–4 μ (Rea).

Gross characters of the rot. *S. purpureum* is one of the principal fungi responsible for the discoloration of felled beech logs, which is known in France as '*échauffure*' and Germany as '*Erstickung*'. Yatsenko-Khmélevky (1938) described the development of this discoloration in the wood of *Fagus orientalis* Lip., stating that it is caused by the reaction of the living cells which, stimulated by the products of fungal metabolism, react by the formation of tyloses and of dark coloured materials of the 'wound gum' type. It has been shown that *Stereum purpureum* produces substances which have a profound effect on the cells of higher plants, for the 'silvering' of foliage may be brought about by substances produced in the branch or trunk at a considerable distance from the affected foliage. The first effect on beech wood of infection by *S. purpureum* is an irregular brown discoloration due to the formation in the parenchyma cell of a gummy material; later small, scattered whitish areas appear giving a mottled appearance. *S. purpureum* does not seem able to cause extensive decay and in laboratory tests has brought about only slight losses in weight, not exceeding 7%, even in perishable woods such as sycamore. Guinier (1933) suggested that *S. purpureum* can only invade wood in which the parenchyma cells are alive and contain reserve materials. The fact that it is usually the first fungus to appear on felled logs in which wood parenchyma cells are still alive, supports this view. He pointed out that it ceases to spread about a year after trees have been felled when the reserve food materials have been exhausted, and that the attack on the cell walls is secondary.

Microscopic details of rot. Hyphae are plentiful in the attacked wood, they are mainly colourless and rather fine, averaging 2 μ diam., but occasionally wide hyphae up to 8 μ across may be observed in the vessels. A few yellow hyphae occur. Clamp connexions, fairly plentiful on young actively growing hyphae, are rarely seen in a later stage of decay. The hyphae penetrate the cell walls forming bore-holes 1·5 μ across.

Fungus in culture. The fungus grows readily on ordinary media. It forms on malt agar a loose colourless cottony growth, which later becomes a closely felted smooth even mat. This may show yellowish or pale fawn tinges, especially at the upper end where in vigorous cultures a small hymenial surface may occasionally be formed. The colourless hyphae from an agar culture bear fairly numerous

rather large clamp connexions which are humped or arched up. Sometimes the clamps occur in whorls at a 'node' and they frequently proliferate. In some of the aerial hyphae the walls are slightly thickened, while ovoid swellings occur on the submerged hyphae.

Physiological data. In culture the fungus grows very rapidly, plate cultures on malt agar showing an increment in diameter of over 2 cm. per day at the optimum temperature which is about 27° C. and the fungus can make some growth at as low a temperature as 3° C. (see Cartwright & Findlay, 1934). Brooks & Storey (1923) found that the spores retain their viability for many weeks in a humid atmosphere but lose it rapidly under dry conditions.

The enzymes of the fungus were studied in detail by Mayo (1925), who found diastase and invertase together with other hydrolysing enzymes and oxidase to be present. Lutz (1934) followed the course of decomposition of cotton cellulose by *S. purpureum* and identified some of the intermediate products and sugars which are formed. Bavendamm (1928) showed that the fungus can tolerate fairly high concentrations of tannin and will grow in a meat and malt extract agar containing over 12% of oak-wood tannin.

Economic importance. As a cause of disease in fruit trees *S. purpureum* is of first-class importance, but as a cause of rot in felled timber it is not so important. It is responsible for the disfigurement of beech logs and for a certain amount of incipient decay in stored aspen logs, but it seldom renders timber unusable except for purposes where maximum strength and/or an unblemished appearance are essential. Rue *et al.* (1924) stated that aspen wood infected with this fungus after a year's storage gave approximately the same yield of pulp as sound wood. Owing to the rapidity with which the fruit bodies appear on felled timber and their rather conspicuous appearance, this fungus receives much attention and is probably credited with causing more damage to timber than is actually the case.

Prevention of infection of fruit trees depends on avoiding as far as possible the formation of wounds: pruning of large branches on plum trees should be avoided, and any wounds which have to be made should be dressed immediately with an antiseptic coating (see p. 250). If felled beech logs have to remain for some months in the woods they should be given an end coating with an antiseptic such as creosote immediately after felling. It is difficult to prevent aspen logs becoming infected with this fungus, and the best way to store them is to keep them submerged in a pond until they are required, but if water storage is not available protective end coatings can usefully be applied.

Ustulina vulgaris Tul.

Synonyms. *Ustulina vulgaris* is now generally regarded as the temperate form and *U. zonata* Lév. as the tropical form of the same species of fungus, and since the former name is the older, *U. zonata* becomes a synonym. It is stated to be synonymous with *U. denata* Fr.

Occurrence. Including the tropical form—*U. zonata*—the fungus appears to have a world-wide distribution, being common on cocoa, rubber, tea, and many other hosts, in Asia, Australia, Africa and America. In Great Britain it is extremely common on old hardwood stumps and logs, and was shown by Wilkins (1934, 1936, 1939*b*) to be a cause of heart rot in lime, beech and elm, and by Blaisdell (1939) in the U.S.A. to be frequent in hardwoods such as maple and oak.

Sporophore. *Ustulina* is a typical Pyrenomycete and a member of the family Xylariaceae. The mature fructification consists of a hard, lumpy, black, stromatic crust dotted with the ostioles of the embedded perithecia. A good description and illustration of the fructification were first given by Tulasne (1863), who established the genus *Ustulina*. The fructifications usually are confluent, and the whole mass may be up to 1 ft. across, giving the impression of a charred area of wood. They are often found underneath the bark or in crevices and hollows in decayed trunks, and sometimes persist for years after they have ceased to function. In each ascus there are eight dark-coloured spores which measure approximately $31 \times 7{\cdot}5\,\mu$ (Wilkins, 1938). The conidial fructification, which under natural conditions precedes the perithecial, is a thin disk, up to 2 in. across, at first bluish grey with a white margin, later becoming yellowish grey and powdery with the ripe conidia. The conidia appear greyish in mass but hyaline under the microscope; they are oval and thin-walled and average $7 \times 3\,\mu$ (Wilkins).

Gross characters of the rot. Detailed description of the rot in lime and in elm was given by Wilkins (1936, 1939*b*), from whose papers the following account is largely taken. Decay starts from a basal wound and progresses into the trunk as a butt rot for 5–10 ft. and also works down into the larger roots. In light-coloured woods without obvious heartwood such as lime and beech, there is a broad red-brown invasion zone due to the infiltration of the cell walls with colouring matter: in elm this is absent or less conspicuous (see Pl. 5, fig. *d*). The decayed wood, which is lighter in weight and paler

than the normal, is bounded by, and frequently (in beech and lime) permeated with narrow, dark zone lines (see Pl. 5, fig. *c*). These black lines are invariably present in wood decayed by *Ustulina*.

Microscopic details of the rot. Fine hyphae about 1 μ wide are present in the decayed areas and in the discoloured area in lime. As decay progresses these become more numerous, till in the final stage of the rot the wood is permeated with a weft of fine mycelium. The black lines consist of a mass of amorphous black material in which it is difficult to distinguish any hyphae. On the outside of a dark line limiting a decayed area, dark-coloured hyphae, about 4 μ across, grow out from the dark line, extending for about 0·5 mm. beyond it. The hyphae pass through the cell walls by means of the pits, the larger ones sometimes enlarging the bordered pits. The first stage in the attack appears to be the gradual disappearance of the infiltration products from the vessels and fibres and, later, from the parenchyma. Then delignified spots appear in the cell walls, and these spread until only the corners of the cells give any lignin reaction. The fibres are the first elements attacked, and the vessels, wood parenchyma surrounding them, and the medullary ray cells persist apparently unchanged till a late stage of decay.

Fungus in culture. At first a white, smooth, flat, even, silky mat is formed which becomes grey at the centre and gradually darkens to black, the coloration spreading outwards. Finally, a hard, black, brittle skin is formed, and this is often deeply depressed at the centre or traversed with radiating ridges and furrows owing to the lateral pressure exerted by the expanding mat. The young hyphae are mostly very fine (under 1 μ) but occasionally hyphae up to 5 μ may be found, the latter being much vacuolated. Branching is frequent and short fusion branches join neighbouring hyphae. Mature hyphae are 4·5–8 μ across. The black skin consists of a definite tissue or pseudoparenchyma of rather thick-walled hyphae. The colour appears to diffuse out and not to be due merely to the colouring of the hyphae walls.

Physiological data. Wilkins (1938) found that the ascospores germinate readily in water and in aqueous extracts of beech and other woods, 25–30° C. being the optimum temperature for germination. Conidia also germinate readily in water and in extracts of wood. Their optimum temperature is lower than for ascospores, i.e. 20–25° C., and he found that they also germinate more rapidly at low temperatures than do the ascospores. He suggested, for this reason, that the conidia are more likely to cause infection than the ascospores. The optimum *p*H for the germination of the ascospores was 6, and for the conidia 5–6. The vitality of the spores decreases fairly rapidly, and the viable percentage steadily drops till it reaches zero, about 6 months after their formation.

Blaisdell (1939) showed that the fungus can cause considerable losses in weight, 26% loss being recorded in black oak samples exposed to the fungus for 6 months at room temperature. Campbell & Wiertelak (1935) analysed lime wood decayed by *Ustulina*, and found that it causes a typical white rot in which the lignin as well as the carbohydrates are attacked, and that the alkali solubility decreases steadily as decay proceeds. Even in the reddish brown invasion zone, some depletion of cellulose occurs, though the wood shows no evidence of rot, and there is an indication that these coloured by-products formed in the early stages of decay are ultimately decomposed. At the most advanced stage which they examined, about one-third of the cellulose and one-quarter of the lignin were decomposed.

Economic importance. At one time the tropical form *U. zonata* was regarded as a dangerous parasite on a number of tropical trees and shrubs, but recently the tendency has been to consider it as of secondary importance, an attack by *Ustulina* often appearing to be associated with infection by some other fungus. The temperate form has long been known as a common saprophyte on stumps, but it was not until Wilkins's investigation showed that the fungus can bring about heart rot in trees such as lime, elm and beech that its importance as a cause of butt rot in temperate trees was recognized. As far as evidence is available, the fungus is only a wound parasite, and its attack follows some damage such as a fire scar, which lays the heartwood open to attack. The fungus can probably continue to spread in felled timber so long as the moisture content is sufficiently high.

The prevention of decay in standing hardwood trees

Since practically all the fungi which cause heart rot in standing broad-leaved trees are wound parasites, the prevention of heart rot is almost entirely dependent on the avoidance of wounds, particularly of large wounds which expose heartwood. Serious wounds are most likely to occur in trees possessing large heavy side branches, and the form of the tree

is thus an important factor in determining its liability to injury. The shape of trees grown in high forest depends very largely on silvicultural practices. Speaking generally, trees which are grown at the correct spacing during their development do not produce large side branches and should, under good conditions, develop a clean bole bearing a well-balanced crown. The small side branches which develop during the growth of the tree die owing to lack of light and fall off before they are large enough to leave an appreciable wound which exposes the heartwood. The wider silvicultural considerations involved in this connexion are outside the scope of this paper, and the following notes deal with other types of damage, their prevention and treatment.

Butt rot caused by *Armillaria mellea* or *Fomes annosus* is usually due to infection entering through the roots which may have been weakened or killed by unfavourable soil conditions, such as bad drainage, the existence of a hard pan or to mineral deficiencies. It has been suggested that infection with *Fomes annosus* may result from rabbit damage to the roots.

Butt rot in elm caused by *F. ulmarius* is often associated with wounds made by deer, and there is no doubt that these animals are very destructive to parkland trees. Hares and rabbits frequently cause damage to trees, such as sycamore and beech which possess thin barks, and horses often strip the bark off trees such as lime or horse chestnut.

Felling operations are often the cause of many wounds to the trunks and exposed roots, and this type of damage is likely to become more severe in the future with the use of heavy tractors in place of horse teams. As far as is known, few attempts have been made in this country to apply any treatment to this type of wound, but it would seem worth while brushing over with a cheap antiseptic, such as creosote, damaged places in the bark of potentially valuable trees, though this would be practical only in the case of wounds near the base of the trunk.

The practice of raising trees from coppice shoots growing from the old stumps of trees such as sycamore, ash, beech, etc., leads to much infection by heart-rotting fungi such as *Ustulina vulgare*. Lorenz & Christensen (1937) stated that in 85% of the cases of decay studied in unburned sprout oak stands in the eastern States of America, the source of infection was traced directly to the parent stump. From a pathological viewpoint it is much better to raise trees from seedlings than from coppice shoots developing from an already infected old stump. There is less risk in the case of the more resistant species such as sweet chestnut, especially where the coppice shoots are to be utilized in the pole stage, than with those species in which the timber has a low resistance to rot.

Rot in the crown or upper part of the trunk is almost invariably the result of infection which has entered through a stub or branch, broken as the result of a gale, of lightning or of an ice storm. Much damage of this kind resulted from the exceptionally severe, glazed frost of the 1939–40 winter, and it is probable that this will lead to heart rot in many of the damaged trees.

Except in specimen trees little can be done to mitigate the effects of such damage in the crowns, but the removal of damaged side branches is a practicable proposition in a managed forest, and is often carried out on the Continent. When removing side branches it is most important that they should be cut off *as close to the trunk as possible* so that callus may grow from the surrounding cambium and heal over the wound. A vertical cut is not so liable to hold water as a horizontal or sloping one. The practice of 'lopping off' branches at some distance from the trunk is strongly to be deprecated.

Many different substances have been used with varying degrees of success for dressing tree wounds. A satisfactory substance for this purpose should be waterproof, fungicidal and to a certain degree, flexible; at the same time it should not injure the surrounding cambium. Collins (1934) gave a useful conspectus of materials which have been used successfully. He stated that liquid grafting wax is particularly satisfactory for scars less than $\frac{1}{2}$ in. in width on choice trees or shrubs, or on scars that are susceptible to injury from tar or creosote. For larger wounds a mixture of creosote and tar has been extensively used with good results, but a mixture of equal parts of melted asphalt and creosote is said to be better. Asphalt alone is very good, but has to be applied hot. Some trees, particularly *Prunus* spp., *Magnolia* and *Liriodendron*, are sensitive to creosote; on such trees it is better to sterilize the wounds with 1/1000 mercuric chloride and then to cover them with paint or spar varnish. On orchard trees a mixture of Bordeaux powder with raw linseed oil has been used with considerable success and should be worth trying on shade trees. It is advisable to examine the dressed surfaces at intervals and to recoat the wounds if there is any sign of cracking or blistering.

Summary

The principal fungi which cause decay in the more common species of British broad-leaved trees are listed with notes on their occurrence and relative importance. The results of tests on the natural resistance to decay of various British hardwoods are tabulated. The principal diagnostic features of the more important fungi which attack ash, beech, elm and willow, and fallen hardwoods generally, are given in a series of tables. Detailed descriptions of the undermentioned fungi, of their appearance in culture and physiological characteristics are given, together with notes on the rots which they bring about, and any available information about their economic importance: *Armillaria mucida*, *Daldinia concentrica*, *Fomes fomentarius*, *F. ulmarius*, *Ganoderma applanatum*, *Pleurotus ostreatus*, *Polyporus betulinus*, *P. cuticularis*, *P. giganteus*, *P. hispidus*, *P. radiatus*, *P. squamosus*, *Polystictus versicolor*, *Poria obliqua*, *Stereum purpureum* and *Ustulina vulgare*.

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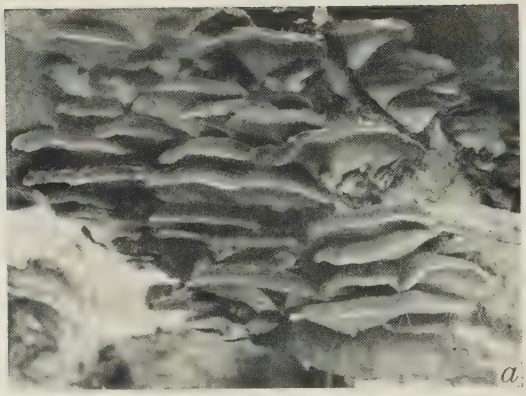
CARTWRIGHT AND FINDLAY—PRINCIPAL DECAYS OF BRITISH HARDWOODS



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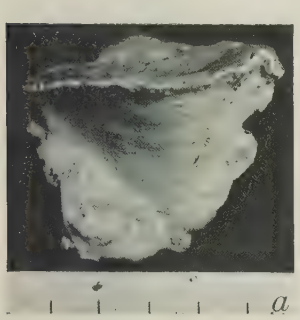
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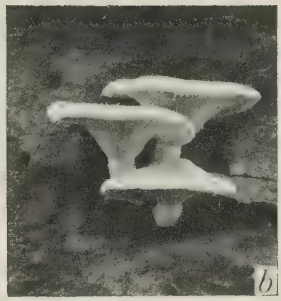
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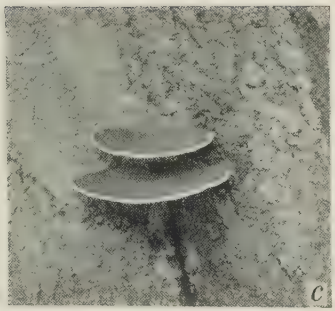
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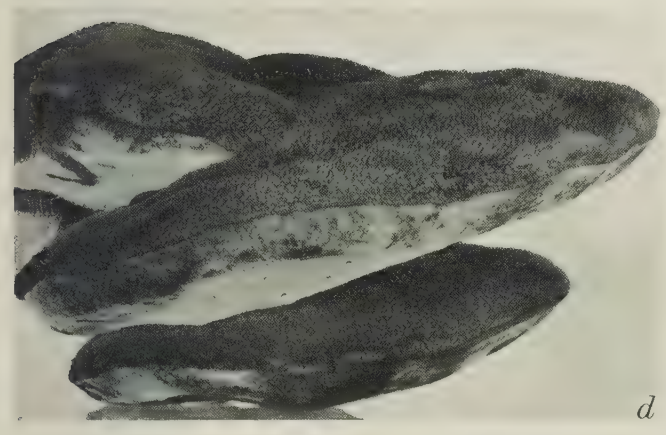
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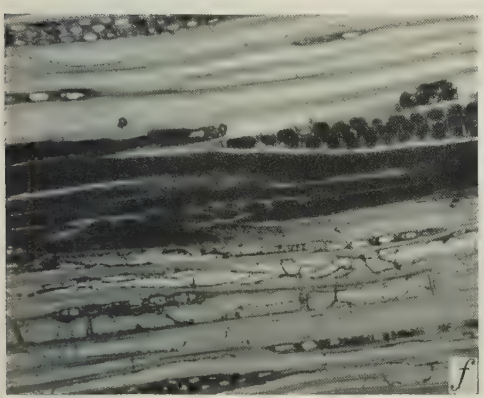
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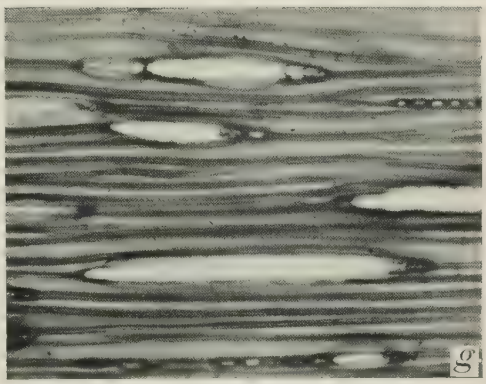
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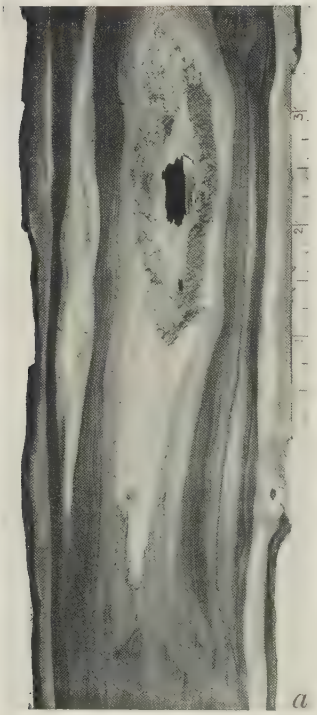

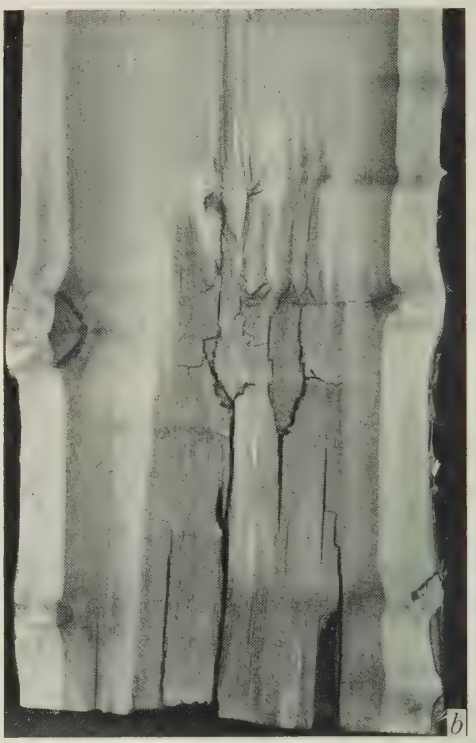

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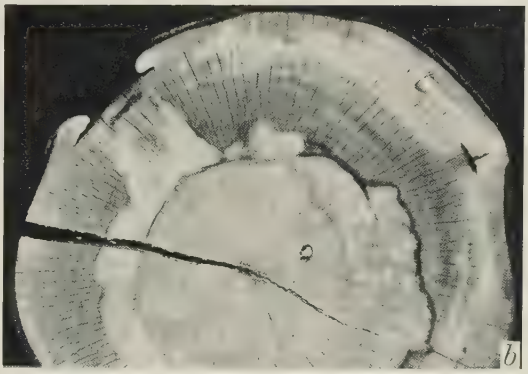

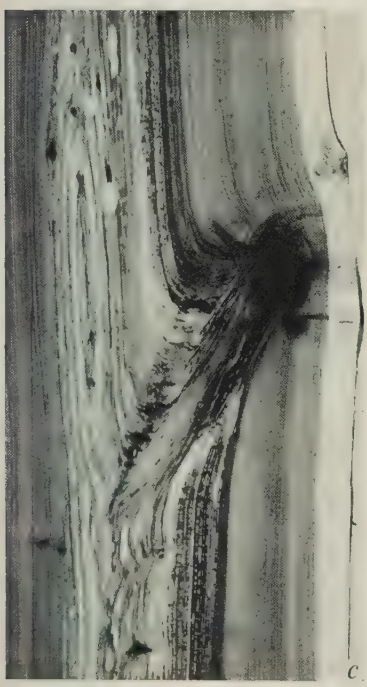

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EXPLANATION OF PLATES 2–6

Plate 2

Mature fructifying cultures on agar of:

(*a*) *Pholiota heteroclita* on 2 % malt.
(*b*) *Lenzites betulina* on 5 % malt.
(*c*) *Fomes ulmarius* on prune agar.
(*d*) *Trametes rubescens* on 5 % malt.
(*e*) *Ganoderma applanatum* on 5 % malt.
(*f*) *Trametes gibbosa* on 12 % malt agar.
(*g*) *Polyporus squamosus* on 5 % malt agar.
(*h*) *Polyporus radiatus* on 5 % acid malt agar grown in light.
(*i*) *Polyporus cuticularis* on 5 % acid malt agar grown in dark.
(*j*) *Pholiota squarrosa* on sterilized wheat grains.

Plate 3

Sporophores of wood rotting fungi in situ:

(*a*) *Polyporus cuticularis* on beech log. × $\frac{1}{4}$ approx.
(*b*) *Ganoderma applanatum* on beech tree. × $\frac{1}{8}$ approx.
(*c*) *Fomes ulmarius* on elm. × $\frac{1}{6}$ approx.
(*d*) *Fomes pomaceus* on *Prunus* sp. × $\frac{1}{8}$ approx.
(*e*) *Fomes fraxineus* at base of poplar. × $\frac{1}{7}$ approx.
(*f*) *Fomes fomentarius* on birch. × $\frac{1}{4}$ approx.

Plate 4

Sporophores of wood-rotting fungi:

(*a*) *Polyporus tephroleucus* (scale in $\frac{1}{2}$ in.).
(*b*) *Pleurotus ulmarius* on elm log. × $\frac{1}{3}$.
(*c*) *Trametes rubescens* on birch. × $\frac{1}{5}$.
(*d*) *Polyporus hispidus*. × $\frac{2}{3}$.
(*e*) *Armillaria mucida* on end of beech log. × $\frac{1}{3}$.

Longitudinal tangential section of birch wood infected with *Poria obliqua*. × 230.

(*f*) Across a zone line showing hyphae massed on inside of line.
(*g*) Showing total destruction of medullary rays in late stage of attack.

Plate 5

(*a*) *Polyporus squamosus*, large rot pocket in elm (scale in ft.).
(*b*) *Fomes ulmarius*, butt rot in elm. × $\frac{1}{4}$.
(*c*) *Ustulina vulgaris*, butt rot in beech.
(*d*) *Ustulina vulgaris*, butt rot in elm.

Plate 6

(*a*) *Ganoderma applanatum*, rot in beech showing narrow dark invasion zone. × 1.
(*b*) *Pleurotus ostreatus*, rot in beech. × $\frac{1}{4}$.
(*c*) *Pholiota squarrosa*, decay in elm. × $\frac{1}{3}$.
(*d*) *Fomes pomaceus*, heart rot in plum. × 1.

(*Received* 8 *January* 1942)

CERCOSPORELLA HERPOTRICHOIDES FRON., CAUSING EYESPOT OF WHEAT IN GREAT BRITAIN

By MARY D. GLYNNE, *Rothamsted Experimental Station, Harpenden, Herts*

(With Plate 7)

Sprague (1931) showed that the fungus *Cercosporella herpotrichoides* Fron. was the cause of a serious wheat disease which had been noted for some years in parts of Europe and America. The disease has now been recognized in a number of countries and was first recorded in England in fields of the Rothamsted Experimental Station Farm, Harpenden, in 1935 (Glynne, 1936). It was found in lodged crops on these and on neighbouring fields as well as in widely separated districts in the southern half of England in the wet summer of 1937, when its importance as a factor in producing lodging in this country began to be suspected. It has since become clear that the well-known kind of lodging which is seen in patches or large areas, here called 'general lodging' (Pl. 7, fig. 4) may occur in heavy healthy crops subjected to strain by winds and storms, but it occurs in less heavy crops and with less strain when they have been weakened by fungal attack. Light crops seldom show general lodging even when a high proportion of the culms is infected, but close inspection before harvest often shows individual straws lying criss-crossed among the upright ones. As many as 75% of the straws may have fallen without the crop appearing lodged. This condition is also seen in heavy crops which are infected without showing general lodging. It will be referred to as 'individual straw lodging' (Pl. 7, figs. 2, 3).

Common name for the disease

The disease caused by *C. herpotrichoides* has been called by such names as eyespot, footrot, strawbreaker and lodging in different countries. In England 'eyespot lodging' has been used (Glynne, 1939), eyespot being descriptive of the characteristic dark-bordered lesions produced on the host near ground level (Pl. 7, fig. 1), and general lodging having been regarded as its most important effect on the crop. Further experience showed that this name puts too much emphasis on lodging and that the disease causes serious loss in other ways; by killing or dwarfing tillers infected early in the season, by the production of white or deaf ears, and by reducing the size and number of grains and from individual straw lodging. As all these effects are accompanied by the characteristic lesions, the less cumbrous name 'eyespot' seems better for the disease, while the term 'eyespot lodging' can be used to distinguish lodging caused by the fungus from other types such as non-parasitic lodging.

History of eyespot in England

Although the fungal nature of the disease has so recently been recognized in this country, there is reason to believe that it has been present for a long time. Some of the symptoms described by Worthington Smith (1884) under the name of 'straw blight' were probably those of eyespot, though he does not mention lodging; others were probably those of take-all, and it is likely that he found both diseases but did not distinguish between them.

On Broadbalk Field, Rothamsted Experimental Station, where wheat has been grown continuously since 1843, 'lodging' and 'straggling' were first recorded in the exceptionally wet summer of 1852, in the ninth successive wheat crop. Since then 'lodging' in some of the plots with the heavier crops and 'straggling' in those with lighter crops, have frequently been recorded in the harvest notes, both often being associated with 'blighted' ears. These terms are still in use applied to the effects now found to be produced by eyespot, i.e. general lodging in the heavier crops and 'straggling' or individual straw lodging in the lighter crops. It is probable that the word 'straggling' has been used consistently and that eyespot causing both 'straggling' and 'lodging' has been appreciable on Broadbalk at least since 1852.

A survey of wheat crops in parts of England in 1941 gave further evidence of the likelihood that eyespot has long been responsible for serious losses in wheat yield in the east and south of England. Individual straw lodging and thin crops resulting from eyespot infection were seen in many districts. Farmers have long been familiar with such trouble for which they still use old dialect terms such as straggling, scrailing, scrawling, shakely, brackley, and possibly also knee-bend, knee-sick, and knee-hapsed (Wright, 1898). Most farmers attributed the condition to too frequent wheat growing and few realized that the cause might be fungal, but eyespot was found to be prevalent in all the cases examined. In some places the terms were used to cover general lodging as well as individual; many farmers distinguished between general lodging caused by disease and that due only to heaviness of the crop. They recognized that non-parasitic lodging, though it adds to the expense of harvest, is indicative of a yield high enough to cover the extra cost; the crop lies in one direction so can be harvested by cutting one way. But when lodging is of the 'shakely' or 'scrawly' type the yield of grain is seriously reduced, and as the straws lie about in all directions tending to break off at ground level, harvesting is extremely difficult and the yield is insufficient to cover the extra cost. Some farmers have known the trouble for 30 years and more and associate it with certain fields. As the terms they use date back some 200 years it is probable that the disease has long been in this country as one of our most serious though unsuspected causes of loss of wheat.

Symptoms of eyespot

Infection takes place in late autumn and early spring from abundant spores produced on stubble lying on the soil. More evidence is needed to show how far infection can be wind borne and how much may occur from the fungus in the soil. Eyespot lesions are commonly found on the host near soil level from March onwards. Secondary infections follow sporing of the fungus on the young plants in March and April. The fungus penetrates the leaf sheaths, dwarfing or killing tillers or even whole plants, so that thin crops may result. But as the disease is favoured by moist conditions, it spreads more easily in a dense crop where a damp atmosphere is preserved near ground level so that the percentage of infection found at harvest tends to be higher in thick than in thin crops. The fungus penetrates one leaf sheath after another, ultimately reaching the culm. This tends to rot, especially under wet conditions, so that any strain makes it bend or even break in the middle of the eyespot. The lesion is on one side of the stem and the straws bend over with a slight twist in the middle of the lesion, so that they fall in all directions; the wind also has an effect on the main direction of fall, especially in general lodging.

Surveys of wheat in 1941

Methods. In 1941 information on the occurrence of lodging and of eyespot in crops of autumn-sown wheat was obtained from three different surveys. Survey I was made by the writer and consisted of car and train journeys from mid-July to the end of August, during which each crop was assessed for the percentage area showing lodging. Every field observable from car or train was assessed by a rapid eye estimation, for the percentage area lodged, which varied from 0 to 90%. During car journeys stops were made every 5 miles or so, the distance depending on the intensity of wheat growing in the particular district. The crops examined were sampled at ten randomly selected spots, 'grab' samples being taken in which counts were made to determine the percentage of straws affected with eyespot. When infection was slight (less than 10%) it could often be detected by looking down the rows and selecting straws individually lodged, which generally showed eyespot; but on occasion wheat stem sawfly produced this type of lodging. The data obtained are given in Table 2.

Fields were grouped according to the estimated percentage of straws diseased as follows:

Straws infected with eyespot %	Severity of infection
0	None
1– 20	Probably has little effect on yield and is chiefly important as a source of infection to a succeeding crop
21– 70	Causes appreciable loss and includes most cases of individual straw lodging in poor crops
71–100	Likely to cause considerable loss in yield, and includes many cases of general lodging

Lodging was estimated for each district by taking the sum of percentage areas estimated as lodged in each field and dividing by the number of fields observed. No allowance was made for the fact that the fields differed in size, but this is unlikely to affect the result significantly. In Hertfordshire where the acreage of fifty-six fields was determined, the percentage area lodged was found to be 4·4 when no allowance was made for acreage, and 4·0 when so corrected. Other fields were visited because disease was suspected, and these, not being randomly selected, were recorded separately and are included in Table 3 with those seen in Survey I.

Survey II, made by Messrs S. D. Garrett and A. G. Walker, was primarily designed as a survey of the incidence of take-all, and the fields examined were mainly first and second wheat crops after ploughing up of grassland. Sampling was done by selecting plants showing whitehead symptoms and examining them at the base for the presence of take-all and eyespot.

Survey III was restricted to Hertfordshire and was made by the Statistical Department of the Rothamsted Experimental Station, to test the accuracy of eye estimations of grain yields. The farms and fields were selected at random, except that whenever possible one of the three fields examined on each farm was newly ploughed grassland. Samples varying from nine to fifty-nine straws were collected from each field by Mr D. A. Boyd. The samples were examined for eyespot but were too small to give any estimate of the amount of infection, only its presence or absence in the sample being regarded as significant.

Comparison of two independent surveys of different randomly selected fields in Hertfordshire can be made by comparing the results of Survey III with those in Survey I. In the former, forty-three out of eighty-one fields sampled had eyespot, i.e. 54%, while in Survey I nineteen out of thirty-six or 53% were infected.

Eyespot in relation to lodging

Table 1 summarizes the records obtained from all fields, whether randomly selected or not. It shows that the percentage area lodged increases with percentage infection by *Cercosporella*, by far the largest increase being in the group showing over 70% infection. In 167 fields with less than 70% straws infected, only 13% had more than 15% of their areas lodged, whereas in twenty-two fields with more than 70% straws infected 68% had more than 15% of their area lodged. The difference is highly significant, showing an undoubted

correlation between eyespot infection and the likelihood of lodging. That lodging is not dependent on infection alone is shown by its absence in some severely infected crops and its occurrence in others which are free from serious infection. The probability of lodging occurring is, however, greatly enhanced by the incidence of a high percentage (over 70%) infection by *Cercosporella*.

TABLE 1

Eyespot % straws infected at harvest	No. of fields	Percentage fields grouped according to percentage area estimated as lodged — % area lodged				Average % area lodged*
		0	1–15	16–60	61–100	
0	76	72	24	1	3	3·5
1– 20	53	47	34	9	9	11·6
21– 70	38	32	47	16	5	13·1
71–100	22	18	14	36	32	37·4
Total no. of fields	**189**	**96**	**57**	**20**	**16**	**11·6**

* Estimated from sum % areas lodged (not quoted), divided by number of fields observed.

DISTRIBUTION OF EYESPOT IN ENGLAND AND WALES

Table 2 gives the records of randomly selected fields examined for eyespot and for lodging in Survey I together with those of seventeen fields on three Norfolk farms provided by Mr Eshuis. The proportions of lodging and eyespot infection in fields observed in different counties are arranged in four groups from west to east.

In the most westerly county, Anglesey, very little winter wheat could be found, but the seven fields seen were outstandingly good, upright, even crops, 4–4½ ft. high with well-filled ears, some of the best seen in the country. In spite of storms in July followed by heavy rain almost until harvest there was no lodging, and no eyespot could be found. Little wheat was grown in Carnarvonshire; in Denbighshire there was more, chiefly in the vale of Clwyd. Most of the wheat was the first or second crop after grass; the crops were excellent with well-filled ears. There was hardly any lodging, except in one of the rare fields which had been arable for several years and where 2½ out of 9 acres were lodged, eyespot was found on over 60% of the straws and whitehead or blighted ears were common. The soil was a heavy type of clay, in a low-lying damp district. Moving eastwards increasing proportions of fields showed eyespot, and there was an increase in the frequency of moderate and severe cases of infection. While only one out of twenty-eight fields examined in Wales showed eyespot, the disease was found in sixty out of seventy-one fields in the most eastern group of counties. The percentage areas lodged increased in the same way.

The intensity of wheat cultivation in each county calculated from the percentage of the total area under wheat is shown in Table 2: the latest figures available are for 1939; as previous wheat crops greatly affect the incidence of eyespot, the 1939 wheat acreage is likely to have had considerable influence on the 1941 wheat crop. Table 2 shows that the great increase in the proportion of land under wheat from west to east, 0·17 to 12·89%, is accompanied by large increases in percentage area lodged 1·1–16·4%, and in the proportion of fields with eyespot 3·6–84·5%. The acreage under barley, also likely to affect eyespot incidence, increased in a similar way, the percentage areas under barley in the four districts from west to east being 0·43, 0·84, 4·89, and 10·75. It was considerably less

TABLE 2. *Distribution of eyespot and lodging from west to east*

Group	Counties grouped from west to east	Area under wheat* % of county	Area under wheat* % of district	Randomly selected fields: Eyespot: No. of fields examined	Eyespot: No. of fields infected grouped according to % straws infected: 1–20	21–70	71–100	Eyespot: % no. of fields infected	Lodging: No. of fields examined	Lodging: % area lodged†
1	Anglesey	0·03	0·17	7	0	0	0	3·6	7	1·1
	Carnarvonshire	0·02		4	0	0	0		4	
	Denbighshire	0·35		17	0	1	0		17	
				28	**0**	**1**	**0**		**28**	
2	Shropshire	3·38	4·19	9	2	1	0	26·3	23	3·2
	Staffordshire	3·56		3	0	0	0		24	
	Warwickshire	4·21		—	—	—	—		21	
	Leicestershire	4·03		2	0	0	0		7	
	Buckinghamshire	4·02		2	0	0	0		3	
	Northamptonshire (ex Soke of Peterborough)	6·46		3	1	1	0		10	
				19	**3**	**2**	**0**		**88**	
3	Soke of Peterborough	12·45	11·89	3	1	0	1	61·5	15	6·9
	Bedfordshire	11·31		5	2	2	0		36	
	Hertfordshire	11·49		36	10	7	2		56	
	Huntingdonshire	16·59		3	1	2	0		38	
	Essex	11·07		5	1	1	2		4	
				52	**15**	**12**	**5**		**149**	
4	Lincolnshire (Holland division)	18·50	12·89	14	9	2	0	84·5	61	16·4
	Cambridgeshire	19·07		31	10	10	5		50	
	Norfolk	9·13		26	13	5	6		47	
				71	**32**	**17**	**11**		**158**	
	Total			**170**	**50**	**32**	**16**	**57·6**	**423**	**9·3**

* Calculated from *Agricultural Statistics* (1930).

† Estimated from sum percentage areas lodged divided by number of fields observed.

than that of wheat in the English counties observed except in Norfolk where it reached the unusually high figure of 14·13.

Eyespot was recorded between 1937 and 1940 in many of the counties in which it was again found in 1941 and in Berkshire, Dorset, Hampshire and Devon. Hitherto no records of the disease have been obtained farther north than Denbighshire and Nottinghamshire, but the northern counties have not yet been surveyed systematically for eyespot. It is clear that the disease is widespread in the southern half of England, being most abundant in the districts of most intensive wheat cultivation.

Eyespot in relation to previous crops

Wheat and barley are susceptible to eyespot but oats show considerable resistance; little is known about the susceptibility of British wild grasses. Spring wheat and spring barley become infected, but the attack is too late to do serious damage though it may be important in carrying on the infection to subsequent crops. Several records of winter barley badly affected in the seedling stage have been recorded since 1937.

The data available from the three surveys on the effect of previous crops on eyespot incidence is shown in Table 3. Crops examined because disease was suspected and those in which it was reported by other observers are included in Survey I · , so that the proportion of diseased crops is rather higher than in the random selection shown in Table 2. Arable crops have been grouped according to the known frequency of wheat and barley during the preceding 4 years. Some records are, however, incomplete as in Survey III and sometimes in Survey II, only the 1939 and 1940 arable crops were noted.

There was no instance of eyespot exceeding 20% in fields where grass or arable without wheat or barley had been grown in the preceding 4 years, and the proportion of these fields showing eyespot was much less than where wheat and barley had been grown. Infection of 21–70% of the straws occurred in some fields where two successive wheat crops (1940-1) followed grass. Crops with over 20% straws infected and even with over 70% were found among arable fields in which wheat or barley had been grown once, but were more frequent when they had been grown twice in the preceding 4 years as is usual in the Norfolk rotation. The occurrence of a few crops, two with over 70% and three more with over 20% straws infected, where the last crop of wheat or barley had been in 1937 or 1938 is important in considering the possibility of control by rotation. The numbers of fields examined is insufficient to test the relative effects of wheat and barley as preceding crops. There were only three fields in which wheat had occurred more than twice in the preceding 4 years. On Broadbalk, Rothamsted Experimental Station, and Stackyard, Woburn Experimental Station, wheat had been grown continuously, and on Pennell's Piece, Rothamsted Experimental Station, it had been grown in 1937, 1939 and 1940. Broadbalk and Pennell's Piece had over 70% straws infected, but no eyespot was found at Woburn, where the light sandy soil contrasts with the clay loam at Rothamsted.

The influence of rotation on eyespot was further illustrated in East Anglia. In the Lincolnshire Fens where wheat and barley are usually grown infrequently in the rotation (sugar beet, potatoes, wheat or oats or sometimes bulbs), eyespot though generally present was seldom more than slight, but in Norfolk where sugar beet, barley, potatoes or clover, followed by wheat is a common rotation, severely infected crops were much more frequent. Again

TABLE 3. *Eyespot in relation to previous crops (fields not randomly selected)*

Previous crops				Survey I+ No. of fields				Survey II No. of fields				Survey III No. of fields		Fields with eyespot	
					Showing eyespot (%)				Showing eyespot (%)						
1937	1938	1939	1940	Examined	1–20	21–70	71–100	Examined	1–20	21–70	71–100	Examined	Showing eyespot	Total % with eyespot in 3 surveys	Serious and severe % with over 20% infected in Surveys I+ and II
Grass	Grass	Grass	Grass	11	3	0	0	36	3	0	0	11	3	**16**	**0**
Grass	Grass	Grass	A	19	2	0	0	—	—	—	—	5	3	**21**	**0**
A	A	A	A	14	9	0	0	1*	1	0	0	21	9	**53**	**0**
Grass	Grass	Grass	Wheat	1	1	0	0	12	3	3	0	2	0	**47**	**23**
Wheat or barley once, A once		A	A	9	5	3	1	1	0	0	1	—	—	**92**	**54**
A	A	Wheat or barley once, A once		36	14	10	10	6*	3	2	1	24	20		
Wheat or barley twice or wheat once and barley once, A twice				19	3	10	5	2	0	1	1	—	—	**95**	**81**
Wheat 3 or 4 times, A once				3	0	0	2	—	—	—	—	—	—	—	—

A, Arable without wheat or barley.

* No records for 1937 and 1938 in Survey III and where indicated in Survey II, so here previous crops only include 1939 and 1940.

a field in Lincolnshire had been divided into three parts in which mustard, potatoes and barley had been grown in 1940. Wheat grown over the whole field in 1941 showed little infection in the parts following potatoes and mustard but that after barley was badly affected both by eyespot and take-all.

The danger of serious infection by eyespot is thus greatest when wheat or barley occur at short intervals in the rotation, but other factors play a part. Farmers tend to associate the trouble with certain fields, and although many serious cases occur in the Norfolk rotation, yet this is frequently practised with little or no trouble from eyespot; the fact that eyespot was not found on Stackyard Field, Woburn, where wheat has been grown continuously indicates that other factors, which probably include soil type, played a part in determining the prevalence of disease. The light sandy soil at Woburn, by facilitating drainage, promotes a dry atmosphere round the base of the plant which helps it to slough off surface infections. Take-all, on the other hand, is favoured by this type of soil and is often severe at Woburn, whereas in the heavier soil at Rothamsted, though the take-all fungus is present, the disease is seldom serious.

Little is known as to how long the disease persists on land which has had a badly infected crop. In two fields (Norfolk and Cambridge Fens) which had severely infected, badly lodged wheat crops in 1939, followed by roots in 1940, the wheat crops grown in 1941 had over 90% straws infected and were laid flat. Such evidence as is available suggests that several years are needed for a badly infected field to recover; one farmer stated that 5–6 years under crops other than wheat or barley sufficed in the case of one of his fields. The time taken will probably depend on several factors such as season, locality and soil type. This is likely to apply also to the rapidity with which fields where wheat is grown continuously become infected. A case is recorded in Norfolk, by Mr Eshuis, of reclaimed marsh land which has grown three successive crops of wheat; the first crop showed no sign of eyespot, in the second it was slight, while the third showed severe infection and was laid flat. Oort (1936) records that in the newly drained Weiringermeerpolder, eyespot was found for the first time to a slight degree in the fourth year of cultivation. At Rothamsted, Pennell's Piece grew wheat in 1934 and 1935 and had less than 5% straws infected in the 1937 wheat crop; it had beans in 1938, followed by wheat which had about 30% straws infected in 1939 and over 70% in 1940 and 1941. Its nearness to the heavily infected Broadbalk field may have influenced its rate of infection.

Loss from eyespot

Without controlled experiments it is difficult to estimate loss from eyespot, but it is obvious that this is considerable. Blighted or whitehead ears associated with eyespot are poorer in grain than healthy ones. Farmers have estimated independently that badly infected lodged crops produce about 30% less grain than corresponding healthy crops even when these are laid non-parasitically. In addition, the disease causes increased liability to lodge and so to suffer damage from birds, wetting and sprouting of grain, as well as increasing cost of harvesting. Field experiments at Rothamsted Experimental Station showed that with similar straw yields the grain from a badly infected crop was only about two thirds that of a healthy one. Counts of numbers of grain in weighed samples showed that those from diseased crops were consistently lighter than those from healthy. It is more difficult

to assess the loss from eyespot in thin, poor crops than in heavier ones. Early loss of plants and tillers is more important in thin crops; the subsequent spread of infection, favoured by damp atmosphere round the plant bases, may be greater in more luxuriant crops, so that they may show a higher percentage infection at harvest than do thinner crops which may actually have suffered greater loss. Thus one part of a field in which the crop, much reduced by eyespot, was very poor showed 68% straws infected at harvest, while another part, in which the crop was better had 98% infected. Many instances of poor crops in which loss from eyespot appeared to be severe were seen in 1941. The severity of the losses caused by eyespot and its prevalence in wheat-growing areas suggest that it is one of the most serious fungal diseases of wheat in this country.

Discussion

Loss in yield from eyespot in wheat, which was serious in 1941, is likely to increase as a result of the intensive cultivation of wheat and barley necessary under war-time conditions. The possibility of reducing such losses without decreasing wheat and barley acreage ought to be considered. This acreage can be increased with least danger from eyespot on land where wheat and barley have not been grown in recent years. In wheat-growing districts the disease would almost certainly be reduced by lengthening the rotation, but the occurrence in 1941 of some severe cases of eyespot on land where the last wheat or barley crop recorded was in 1938 and even in 1937, shows that under certain conditions, several years may be needed between wheat and barley crops to avoid heavy infections.

More information is needed on the time taken to free land from heavy infestation by eyespot and to determine to what extent volunteer wheat and barley plants, wild grasses and other possible hosts harbour the fungus. More precise knowledge may indicate the length of rotation necessary to eliminate serious cases of eyespot under different conditions, but while the war-time need to increase wheat production persists such measures may not be in the national interest even on land where the disease has been serious. It seems advisable, therefore, that when wheat is grown on land where eyespot has been severe such methods of control as are likely to reduce the disease should be used. These include measures for reducing atmospheric moisture round the base of the plant, especially in spring when secondary infection takes place; such as good drainage, thin sowing, wide spacing of rows, the choice of sparsely tillering varieties, and the checking of 'proud' spring growth by sheep feeding and, in the Fens, by spraying with sulphuric acid or 'grass-cutting' the crop. The use of short-strawed wheat varieties, which are less likely to lodge in spite of disease infection, has given hopeful results and might also be tried. Records of fields known to have suffered from eyespot might well be kept in all districts and the success or failure of control measures reported.

Summary

Surveys of wheat in 1941 showed that the probability of general lodging in heavy crops is greatly increased when a high percentage of the straws is infected by *Cercosporella herpotrichoides*. Individual straw lodging resulting from infection occurs both in heavy and in light crops, so that the straws fall in all directions. The trouble has been known to farmers for many years under various dialect names, and when severe is estimated to cause a reduction in grain yield of about 30%. A survey of 170 randomly selected fields in sixteen counties showed increases in the frequency and severity of infection by eyespot from west to east, 3·6% of the fields in North Wales showed eyespot and 84·5% in the eastern counties; the percentage area lodged increased in the same way from 1·1 to 16·4% and the percentage of the area under wheat (in 1939) from 0·17 to 12·89%. Two hundred and thirty-five fields not randomly selected, in three independent surveys, included 118 in which no wheat or barley had been grown in the preceding 4 years; thirty-seven of these showed eyespot infection, always less than 20% straws being infected; 115 fields which had wheat or barley recorded at least once in the preceding 4 years, included eighty-nine with eyespot, more than half having over 20% straws infected. Infection of over 20% and over 70% occurred on a few fields in which the last preceding wheat or barley crop had been in 1937 or 1938, but they were most frequent where wheat or barley (or one of each) had been grown in at least 2 of the preceding 4 years. The disease is likely to increase in wheat-growing districts under the more intensive wheat cultivation resulting from war-time conditions. Measures for reducing loss from eyespot, while increasing wheat acreage, are discussed.

The writer wishes to thank Miss W. M. Ritchie, who examined all the specimens received from Survey III, Miss Mary Burton who helped in preparing the Tables, County and Advisory Officers who effected introductions to farmers in their districts, and those who contributed field records, Messrs Garrett, Walker and Boyd for data and specimens from their surveys, and Messrs Eshuis and Richardson for much information from their own and neighbouring farms.

APPENDIX

Extracts from *The English Dialect Dictionary*, edited by Joseph Wright 1898–1905, on words now used for corn lying about untidily as it is when attacked by eyespot:

Straggled (Yks, Ches., Oxf.). Of corn; twisted, laid by storms, etc. (N. Yks) Twinds straggled that standing corn.

Straggelt or *Straggalt*. A thin worthless crop of corn, grass, etc.

Shackle. To lay standing corn. (Rutland) After some heavy rain the corn is 'so shackled that you cannot reap it.'

Scrail also written *Scrale* (Northants). Standing corn or grass beat down irregularly by wind or cattle is said to be scrailed; when thin crop of corn partially stands erect, the part that falls is scrailed. (Beds.) Wheat is said to scrale when it is laid. (Herts.) (Wheat blown down) lay scraled and confused. Ellis, *Mod. Husb.* 1750, 4, 1, 49. Hence scrailly, adj. beaten down irregularly; used of grass, corn, etc. (Northants) So scrailly it can't be reaped.

Scrawly (several counties). Adj. of corn: thin, and entangled by the wind, blown about. A crop of corn that is ripening is said to 'go scrawly' or 'be scrawly' when it is blighted and leans in various directions owing to the unsoundness of the straw. 'If you get that land in too good condition you'll have the wheat go scrawly.'

Brackly (Suffolk). Particularly applied to standing corn, some ears of which are so quickly ripened as to snap off short. 'Ripe corn, especially wheat, is said to brackle when, from having too quickly ripened or from other causes, the stems are brittle, and snap short off under the sickle, or the gleaner's hand.'

Knee-bowed, of corn bent down, laid.

Knee-hapsed, of corn bent down, laid.

Knee-sick, of corn weak in the stalk, drooping from weakness. E.g. W. Somers: 'Thick field of wheat looks knee-bowed like; nif don't hold up soon, he'll go lie altogether.' The term scarcely implies that the crop is completely beaten down—this is 'go lie'.

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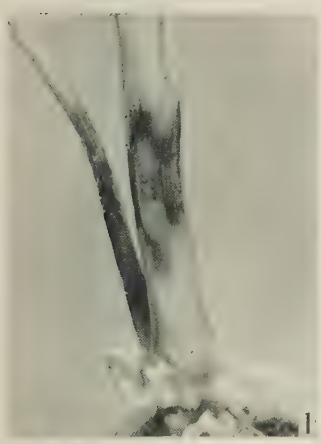
EXPLANATION OF PLATE 7

Fig. 1. Eyespot lesion caused by *Cercosporella herpotrichoides* on wheat in May.
Figs. 2, 3. Individual straw lodging, caused by eyespot in August.
Fig. 4. General lodging caused by eyespot in August.

(*Received* 31 *December* 1941)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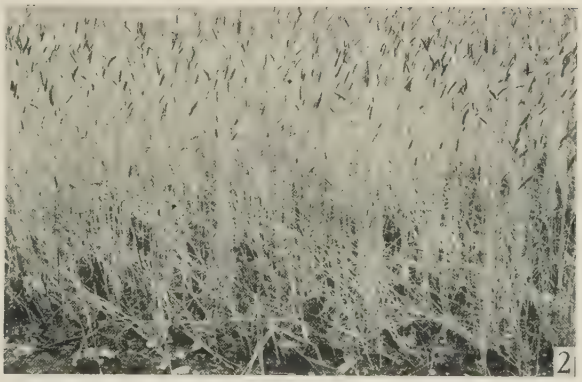
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GLYNNE—*Cercosporella herpotrichoides* Fron., causing eyespot of wheat in Great Britain

THE PRODUCTION OF VIRUS-FREE POTATOES IN THE SOUTH-WEST OF ENGLAND

By JOHN CALDWELL, *Department of Botany, University College, Exeter, Devon*

A preliminary survey in the autumn of 1936 showed that in isolated districts in Cornwall and Devon, potatoes had been grown from seed saved on the same farm for many years. Many of the stocks were relatively free from virus disease, comparing favourably with crops grown from imported seed. One stock examined had been grown on one farm for 15 years and was practically virus-free: actually, the farmer had unconsciously rogued the crop, preferring to take his seed potatoes from plants selected as 'well-grown' while the crop was still in the ground, and so probably eliminating the few infected tubers from year to year. It was evident that areas in the two counties are very suitable for the production of clean stocks of potatoes. Further, crops from seed saved in the area matured earlier than did similar stocks from Scots or Lincolnshire seed, so that from the point of view of early potato production it may be desirable to grow the seed potatoes locally. The difference in earliness of maturing of the crop was 2–3 weeks in some areas, a factor of great economic importance when the price of first earlies is high: the earliness of maturing is not associated with virus disease. Apart from the isolated areas where the chance of infection from other stocks is very slight, much of the area under consideration conforms to the requirements outlined by the late Maldwyn Davies for districts with low aphis counts. The climate is generally humid, often with prevalent mists, and in most districts the wind velocity is high.

In the light of these considerations a survey was made of possible localities—windswept and with a high humidity—and farmers and others were approached with a view to enlisting their co-operation. This was made possible by the kindness and help of the Ministry of Agriculture's Inspectors in the counties. After a general survey of the possibilities of the peninsula, twelve stations were selected for experimentation. The co-operation of a grower progressive enough to help was essential, the area had to conform to the requirements of climate and exposure outlined above, and the sites had to be distributed over the whole of the area so far as was compatible with the two previous requirements.

The conditions required of the growers who participated in the experiment were that they should grow the stocks for not less than 2 years, and that the seed given them should be grown as far as was convenient at a distance from other potatoes during the first year, at least. A distance of 300 yd. was suggested as suitable, but some of the growers were unable to fulfil this requirement, and, almost by accident, it was found that much less than 300 yd. isolation was required in some areas. The purpose of the requirement of isolation of the stocks was simply that it was not known what was the condition of the main stocks which some growers had on their farms, and it seemed undesirable that the clean stocks should be contaminated early in the season before any observations had been made.

Stocks distributed for trial were selected from those available commercially or were obtained by the kindness of the officers of the Plant Breeding Station at Corstorphine and of the East of Scotland Agricultural College in Aberdeen. On the advice of officials of the Ministry of Agriculture the varieties of commercial stocks selected were Sharpe's Express,

Arran Pilot, May Queen, Duke of York, Dargill Early and Arran Consul. Except for the Dargill Early stock, a sample of each group was kept in Exeter for growing under controlled conditions and for purposes of keeping check on the growth of the plants. The remainder of the stock was divided into four lots, and one lot of each of two varieties was sent to each of the co-operating growers. The stocks were sent out before the end of 1936 and were sprouted in boxes and planted at varying dates according to the normal practice in the area. In early spring, 1937, they were examined by me, and in a few instances plants of doubtful appearance were removed from the rows. A small number of plants of some of the stocks definitely was infected with mosaic and these plants were lifted completely and burned, as were a few others in some stocks which did not appear to be growing well. I examined most of the stocks again later in the season, and all of them were examined from time to time by the Ministry's Inspectors. In accordance with their agreement the growers lifted and stored the whole of the crop. At some of the centres the isolation of the stocks was not so complete as had been agreed upon, and they were grown in close proximity to regular commercial stocks on the farm. It was thought desirable to continue with these stocks to discover how far the virus present in the commercial stocks had been transferred to the experimental stocks, a point which would give some idea of the efficiency of the vectors, should any be present.

The presence of aphides in potato stores may be of considerable importance in the spread of virus in this area, where the weather in winter is normally mild, and the growers agreed to ensure that no aphides were present on the stored tubers. It is very probable that one stock, which became badly infected with virus disease was infected while in the store. One grower, who has been particularly successful in maintaining an excellent stock of Sharpe's Express free from disease, reported that he was very careful to examine his stored potatoes to ensure the absence of aphides.

By kindness of Mr J. C. F. Fryer of the Ministry of Agriculture's Plant Pathological Laboratory, it was arranged that Mr Staniland should carry out an aphis count on all the growing stocks which were under trial. He reported aphis counts ranging from nil to only a few on all the stocks but one, a stock growing on a small farm which was primarily concerned with the production of cut flowers and which was grown in a particularly sheltered part of a region which was in the main rather exposed.

The importance of the 1938 crop in indicating the suitability of the centres for seed-potato growing was realized, since it was the amount of the infection which had taken place while the crop was growing during the previous season which would determine to what extent the area was suitable for seed production. All the centres were visited at various times by the Ministry's Inspectors and the health of the plants was noted, as also their general habit, since there is a prejudice in the area against stocks which have a tendency to 'bolt'. The type preferred in the early varieties grown in this area has a low-growing, compact type of foliage; a type highly prized in varieties like Sharpe's Express, May Queen and Arran Pilot. It was noted in these experiments that plants of this type did mature earliest and produced good crops of tubers. On the College Estate it was arranged that not only the health and type of plants should be examined but a comparison should also be made between the time of maturity of a crop from Devon-grown seed and one from imported Scots seed, since it is considered by some workers that the early maturity of crops from seed grown in the south may be conditioned not by physiological changes but

by the presence of virus. For the purposes of the comparison small stocks of the six varieties grown were obtained through a local seed merchant, and planted in rows adjacent to those of the corresponding variety of our own seed in the season 1938. When the plants were examined from time to time throughout the season it was found that the commercial stocks were infected with virus—mosaic, etc. (not leaf roll). There was a considerable difference in the time of maturity of the two stocks, and an interval of about 3 weeks elapsed between the 'ripening'. The plants from Devon-grown seed were consistently ahead of those grown from Scots seed. There was no evidence that the presence of virus was a major factor in inducing early maturity in the stocks. It seems much more probable that physiological changes are induced by the climatic conditions in the area which are reflected in early maturity of the crop.

This aspect of the problem was carried a stage further in the 1939 season. A mixed group of Arran Pilot potatoes, some healthy and some diseased with virus *X* (Solanum Virus I) was planted in the normal manner on 11 Apr. and dug on 23 June when the foliage of the infected plants had become yellow and the plants looked 'ripe'. The healthy plants at the same date were green and clearly would have grown for some weeks. The diseased plants produced a yield of $\frac{2}{3}$ lb. on the average and the potatoes were in the main small. The healthy plants produced a crop of just over 1 lb. on the average, the potatoes were larger in the main, and clearly they would have increased in size had the plants been kept in the ground for a longer period. Although the diseased plants looked more mature, it would have paid on the grounds of yield to have dug the healthy plants which looked much less ripe, when a better crop would have been gathered.

In the course of the experiments some of the growers ceased to interest themselves in the production of seed. In only two cases were the experiments concluded, as the locality and conditions made the production of virus-free stock difficult if not impossible.

Conclusions

It is clear that large quantities of seed potatoes could readily be produced in three main areas in the peninsula, viz. on Dartmoor, or Bodmin Moor, and on parts of Exmoor. Many other areas are available where conditions are satisfactory, and much of west Devon and Cornwall could be used for the production of seed potatoes. In the main those districts which are most suitable for this purpose are those least suited for other forms of agricultural activity, so that this industry would not be prejudicial to other pursuits, and would afford a profitable use for land not now cultivated successfully. Some elementary precautions would need to be taken: (1) varieties must be grown in isolation: there is a tendency to grow a number of different varieties in one field and usually one or other is heavily infected; (2) the custom of allowing farm workers to grow a few rows of their own seed in the middle of a crop should be stopped.

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SYMBIOSIS AND SIRICID WOODWASPS

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The siricid woodwasps are well known as insects of considerable economic importance in forestry, and the habits, development and control of the several species which occur in this country were discussed in detail by Chrystal (1928) and Hanson (1939). These wood-borers have an additional interest for biologists, because they live in symbiosis with certain fungi, which are themselves of economic importance as wood-destroyers. Whereas the fungi in question are of common occurrence apart from the insects, the woodwasps, with the exception of *Xeris spectrum* L. (Francke-Grosmann, 1939), have been found only in close association with the fungi.

The symbiosis was first described by Buchner (1928, 1930) and has since been investigated by Cartwright (1929, 1938), Clark (1933), Müller (1934) and in particular by Francke-Grosmann (1939). As a result of these researches, the history of the fungus has been traced almost completely through the egg, pupal and imaginal stages of the insect, but the occurrence of fungus in the larva, other than in the contents of the alimentary canal, has remained uncertain. The aim of the present work has been to confirm and to extend the observations and conclusions of other workers and, especially, to ascertain whether there is a close association between fungi and siricid larvae. Owing to the war it has not been possible to complete the investigation, but this paper gives an account of the work so far carried out. A note on the most interesting result has already been published (Parkin, 1941).

Buchner (1928, 1930) discovered in the female imagines of *Sirex gigas* L. a pair of small invaginated intersegmental sacs, which projected into the body cavity at the anterior end of the ovipositor and were filled with the oidia of a basidiomycete fungus. He found similar structures in several species of the Siricinae and Xiphydriinae, and the list has been extended by other workers. Various species of basidiomycetes have been isolated from the sacs of some of the Siricinae, but Cartwright (1938) reported that he cultured an ascomycete from *Xiphrydia prolongata* Geoffr. The only exception is *Xeris spectrum* L., which was reported by Francke-Grosmann (1939) to possess very small intersegmental sacs devoid of fungal contents.

My investigations have been confined to *Sirex gigas* L. and *S. cyaneus* F., in which dissection from above reveals the intersegmental sacs as a pair of brownish or pinkish ovoid structures lying on the floor of the abdominal cavity at the anterior end of the ovipositor. Sections show that they are thin-walled and have a chitinous lining which is perforated by the openings of numerous long sinuous ducts originating from unicellular glands in the club-shaped proximal ends of the lateral ovipositor stylets. Fungal oidia, consisting of nearly straight lengths of one to four cells on which clamp connexions are usually prominent, fill the cavity of the structure. It is easy to transfer some of the oidia on the point of a sterilized needle to a malt-agar slope and grow a pure culture of the fungus. Francke-Grosmann (1939) concluded from examination of five species of siricids that the individual

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species are not always associated with the same fungus, although a definite fungal species appears to predominate within a single species of woodwasp. Cartwright (1929, 1938) and myself, however, found in the course of many isolations from *S. gigas* and *S. cyaneus* only one species of fungus, identified by Cartwright as *Stereum sanguinolentum* (A. & S.) Fr. There are slight but distinctive differences in the general appearance of the mycelia grown from the two species of woodwasp, but they are insufficient to be classed as more than varietal differences. Clark (1933) isolated from *Sirex noctilio* F. in New Zealand a fungus which he states is identical in culture with *Stereum sanguinolentum*. Since this species of fungus is a white rot of softwoods, other species must be present in woodwasps which attack hardwoods. No fungus has been detected in adult males, and it is evident that the association is confined to the female wasps.

The intersegmental sacs open into the channel of the ovipositor and not into the oviduct. They have a thin superficial musculature, and during oviposition small amounts of the oidia are extruded into the ovipositor so that each egg becomes infected as it is laid. Cartwright (1938) said that the fungus in *Sirex cyaneus* appears to develop definite mycelial growth on the egg before oviposition, but I have not been able to obtain any fungal growth by incubation on malt agar of eggs of *S. gigas* and *S. cyaneus* dissected out of the ovaries or oviducts. On the other hand, I have made strong, pure cultures of *Stereum* from an egg laid reflexly by a female *Sirex cyaneus* during dissection in physiological salt solution, and from eggs cut out of oviposition tunnels in wood. Cartwright (1929) stated that the fungus can be introduced into sound wood through the oviposition tunnels of *Sirex*, a point of obvious economic importance.

In the oviposition tunnel the oidia on the eggs rapidly develop into hyphae which penetrate the surrounding wood and precede the larva in its boring. It is not yet clear how the larva obtains its nourishment from the decayed wood. Buchner (1928, 1930) supposed that the fungus 'predigested' the wood for the insect. Müller (1934) analysed the food wood and frass of *S. gigas* and *S. phantoma* L., and showed that the frass contained less cellulose and pentosans, but in the absence of detailed knowledge of the effect of the preceding fungal attack the conclusion that woodwasp larvae can themselves digest the cell-wall constituents of the wood is not permissible. Francke-Grosmann (1939) is of the opinion that the larvae live on the fungal mycelium ingested with the wood and, in support of this, has shown that hyphae in sections of attacked wood are rapidly destroyed in vitro by the larval digestive juices. This view is supported by Cartwright's (1929) observations that a newly hatched larva was able to live, and apparently feed, for 3 weeks on a culture of *Stereum sanguinolentum*, and a half-grown larva lived and definitely fed for 3 months. Although the fungus must play an important part in the nutrition of the larvae, its exact role can be determined only after further experiment.

Cartwright (1938) stated that 'sections across late stage pupae also showed fungus to be present in the glands at the base of the ovipositor in the case of female pupae', but Francke-Grosmann (1939) considered that pupae are sterile with regard to fungi and concludes that the fungus must grow into the invaginated intersegmental sacs from the wall of the pupal chamber after the pupal skin is cast and while the immature adult is hardening. She obtained a female *Sirex gigas* free from fungus by removing it from the wood before the pupal skin had been shed; sister woodwasps, emerging normally, were infected. My observations support Francke-Grosmann's conclusions. I have been unable to detect any fungus

in sections of female pupae and all attempts to culture fungi from the tissues in the region of the developing sacs have failed. In addition, the fungus could not be obtained in culture from a young female *S. cyaneus* cut out of the wood immediately after eclosion from the pupal skin, but was grown without difficulty from a female captured as it was about to emerge from the log. It appears, therefore, that the fungus enters the intersegmental sacs of the adult female woodwasp after the pupal skin has been cast. It must be assumed that the unicellular glands in the heads of the lateral ovipositor stylets discharge into the lumina of the sacs a substance which acts both as an attractant and a food for the fungus. The hyphae probably grow from the wall of the pupal chamber into the sacs where they proliferate until the nutriment is exhausted and then break up into oidia. On the other hand, although I have successfully cultured *Stereum sanguinolentum* from the intersegmental sacs of newly emerged virgin *Sirex cyaneus* females, other females, extracted from their pupal cells before the exit-holes were large enough to allow emergence and kept without mating for 8 days, possessed tiny sacs containing scarcely any fungus. This observation requires confirmation before it is concluded that secretion of sufficient fluid to enable the fungus to proliferate does not take place until after the stimulation of mating. Francke-Grosmann (1939) raised the interesting point, whether the intersegmental sacs have developed under the influence of symbiosis or whether they were formed to permit a considerable range of movement of the proximal end of the ovipositor and have only secondarily led to the development of a stable symbiosis.

The symbiotic cycle in *Sirex* can thus be completed without reference to the larva. Clark (1933), however, reported the discovery of organs containing fungus in the region of the hindgut of *S. noctilio* larvae and states that they correspond to those found in the adult female of *S. noctilio*, and are carried from the larva through the pupal stage to the adult. From one individual he obtained a culture of *Stereum sanguinolentum*, but his other attempts at isolation were ruined by contaminants. Müller (1934) and Francke-Grosmann (1939), apparently unaware of Clark's work, stated that, apart from mycelial remains in the gut, no fungi could be detected in sections of larvae at different stages of development.

My investigation into the occurrence of fungi in the larvae of *Sirex gigas* and *S. cyaneus* has yielded results which differ completely from the findings of the three workers mentioned above. Careful examination of serial sections of larvae has disclosed the presence of peculiar epidermal structures containing fungal strands. These structures were sought on the living insect and have been found on some, but not all, larvae. There is one on each side of the body, concealed in the deep fold between the first and second abdominal segments, and formed by local modifications of the cuticle and hypodermis on the posterior sides of the hypopleural folds of the first abdominal segment. By reason of their position they will be referred to as the hypopleural organs. The infolding of the skin between the corresponding two segments of larvae not possessing these structures is noticeably shallower. There is evidence for assuming that the organs occur on female larvae only. When part of an infested log was split 30 larvae and 43 pupae and immature *S. cyaneus* wasps were found: of the larvae 18 possessed hypopleural organs, a proportion of 1 to 1·67, and 25 pupae and wasps were females, a proportion of 1 to 1·72. Unfortunately, larvae kept in Petri dishes failed to pupate, and the sexual significance of the presence or absence of hypopleural organs could not be confirmed. I have been unable to detect any similar organs on first-

stage larvae examined whole or in sections, but they can be seen in larvae one-quarter to one-third grown and become larger as the larva grows.

Microscopic examination of pieces of larval skin, excised so as to include a hypopleural organ, shows (Fig. 1) that in surface view the general outline of the structure is fusiform, the long axis being slightly curved so that the concavity faces the middle line of the insect. The cuticle is pitted in a characteristic fashion, and the size and number of the pits increase as the larva grows. Measurements of the hypopleural organs of nearly fully grown larvae are as follows:

Species	Length	Max. width
S. cyaneus	1·22 mm.	0·25 mm.
S. gigas	0·94 mm.	0·31 mm.

Fig. 1. Surface view of larval hypopleural organ, showing arrangement of pits. A, *Sirex cyaneus*; B, *S. gigas*.

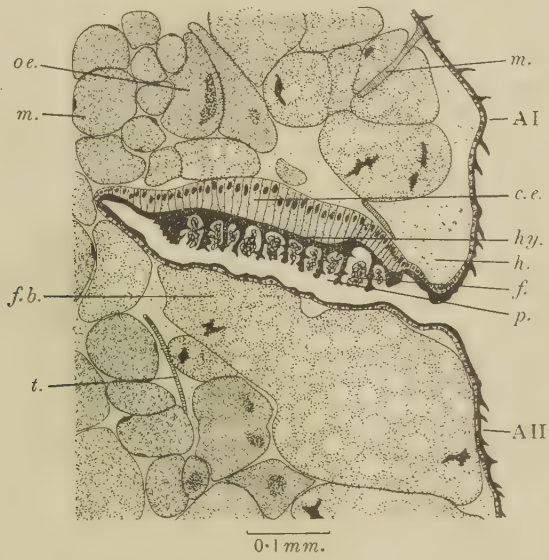

Fig. 2. Part of longitudinal section through *Sirex cyaneus* larva in region of hypopleural organ. A I, A II, first and second abdominal segments; *c.e.* columnar epithelium; *f.* fungal threads; *f.b.* fat body; *h.* haemocoele; *hy.* hypopleural organ; *m.* muscle; *oe.* oenocyte; *p.* pit in cuticle; *t.* tracheole.

The different proportions of length to width makes it possible to distinguish the hypopleural organs of the two species. Examination of a siricid larva of unknown species, found in a log of softwood imported from Poland, showed that, although the organ was of similar shape to that of *S. gigas*, the individual pits were very small and much more numerous in each transverse row. It is believed that the hypopleural organs may be used not only to indicate the sex of siricid larvae, but also to identify the various species.

In section (Fig. 2), the organ appears as a series of deep recesses in the thickened cuticle, the walls of the recesses being furnished with spines. These spines serve to retain within each pit a mass of fungal threads so closely intertwined that it has not been possible to determine whether the fungus is present as hyphal filaments or oidia. Clamp connexions

have not been observed, but this may be a fault of the staining technique which was directed to differentiation of the insect tissues. The underlying hypodermal cells are greatly enlarged to form a columnar epithelium which, no doubt, has a special secretory function. It is not known whether this hypertrophy is connected with secretion of the complex cuticular structure at each moult or whether the cells provide a nutrient material for the fungus.

In order to discover the identity of the fungus, larvae of *S. gigas* and *S. cyaneus* were immersed for a few seconds in 95 % alcohol and dried on a filter paper previously sterilized in alcohol. A small piece of skin including a hypopleural organ was dissected off, freed from adhering muscles, fat and tracheae, and planted on a slope of malt agar in a test-tube: all instruments used in these operations were sterilized in a flame or in alcohol. The tubes were maintained at 22° C. and, after a few days, a strong pure growth of *Stereum sanguinolentum* was observed in the majority: the identity of the fungus was confirmed by Cartwright. In the remaining tubes growth of this fungus was often visible before it was swamped by contaminants. Isolation of *Stereum* was particularly difficult when larvae were cut from wood heavily infected with *Trichoderma lignorum* (Tode) Harz.

Discussion

In spite of recent advances in knowledge of the symbiosis between siricid woodwasps and wood-destroying fungi, there are a number of puzzling features requiring further investigation. There is still doubt as to whether each species of woodwasp is associated with a particular species or variety of fungus. Francke-Grosmann (1939) considered that the relation is not so specialized, but Cartwright (1929, 1938) and myself have never isolated from *Sirex gigas* or *S. cyaneus* any other fungus than *Stereum sanguinolentum*, except as a contaminant. We have independently isolated this fungus on many occasions and at considerable intervals of time from adults, eggs, oviposition tunnels, and larval galleries. In addition, I have regularly isolated the same species from the hypopleural organs of half to fully grown larvae. It therefore seems that this species of fungus is the only one in this country associated with the two woodwasps mentioned: in this connexion Clark's (1933) isolation of the same fungus from *Sirex noctilio* in New Zealand is of particular interest. Finally, the case for some degree of specificity is supported by Hanson's (1939) observation that *Sirex* larvae never burrow in wood penetrated by *Fomes annosus* Cooke and often die when tunnelling through wood decayed by *Armillaria mellea* Vahl., although trees killed by either fungus are attractive to adult wasps for oviposition.

Buchner (1928, 1930) emphasized the close association between woodwasps and fungus, which he supposed had resulted in the development in the adult female wasp of special organs for storage of the fungus and for its transmission to the eggs during oviposition. Francke-Grosmann (1939) suggested that the intersegmental sacs were originally developed as lubricating organs for the basal parts of the ovipositor and that they have become regularly invaded by fungal hyphae growing from the wall of the pupal chamber during the quiescent period between the casting of the pupal skin and the emergence of the hardened wasp. Neither view, however, seems to offer any explanation of why the fungus should occur in special organs on a proportion of the larvae. Nothing is yet known about the fate of the larval hypopleural organs during moulting, but, as that part of each organ which actually contains the fungus is a cuticular structure, the association must presumably be broken each time the skin is cast. If this is so, it must be assumed that the hypopleural

organs of the newly moulted larva are reinfected by hyphae growing in from the wall of the tunnel in the wood. One may well ask, what attracts the fungus to grow into the organs and what can be the value of the fungus to the larva when the association is broken and must be re-established at each ecdysis? Also, why does the fungus occur only in some larvae, probably the females? Since the remaining larvae exist without fungus in bodily association, the contents of the hypopleural organs can apparently have no direct influence upon growth or metamorphosis, especially as they are virtually external to the body of the larva.

In the absence of fungus from the pupa, as shown by the observations of Francke-Grosmann (1939) and myself, it becomes all the more difficult to understand why female larvae should develop a pair of special structures in which the hyphae are intermittently stored. The fungus seems to be unnecessary to the larva except in the wood surrounding the gallery, where its presence has, no doubt, a nutritional significance. The mycelium may itself be the chief food of the larva or it may render the wood more available or more easily digestible for the insect. The larval gut, however, is much simpler than the types generally found in larvae digesting wood and Cartwright (1929) and Francke-Grosmann (1939) produced evidence to support the view that siricid larvae are mycetophagous. In either case, why should only *Stereum sanguinolentum* be associated with *Sirex gigas* and *S. cyaneus* (and perhaps *S. noctilio*), when one might think that any other fungus of similar type attacking softwoods would serve the insect equally well? The answers to these questions may be forthcoming when the investigation can be resumed.

Summary

A reinvestigation of the association between fungi and the woodwasps, *Sirex gigas* L. and *S. cyaneus* F., showed that a single species of fungus, *Stereum sanguinolentum* (A. & S.) Fr. is present in the intersegmental sacs situated at the anterior end of the ovipositor of adult females. The egg becomes infected with fungal oidia at the start of its passage down the ovipositor. When the egg has been deposited in timber, mycelial growth commences and the fungus subsequently precedes the larva in its boring. The larva is probably, at least in part, mycetophagous. Modifications of the larval integument on the posterior aspect of the first abdominal segment have been discovered. The structures so formed, termed hypopleural organs, contain fungus which has been cultured and identified as *Stereum sanguinolentum*. The organs are found in a proportion of the larvae, which suggests that they occur in female larvae only. The fate of the larval hypopleural organs during ecdysis or at pupation is unknown, nor can the importance of these organs in the symbiotic cycle be assessed. No fungus could be detected in pupae and it is thought that the fungus must grow from the walls of the pupal chamber into the intersegmental sacs of the immature female immediately after emergence from the pupal skin.

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SOME NOTES ON THE BIOLOGY OF THE CLICK BEETLES *AGRIOTES OBSCURUS* L. AND *A. SPUTATOR* L.

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Wireworms rarely occur in permanent arable land in sufficient numbers to cause serious damage to crops. Various workers (e.g. Roebuck, 1924; Hawkins, 1936; Miles & Cohen, 1941) have noted the reduction in numbers of wireworms in successive years on ploughed-out grassland. At Rothamsted reductions of 62% in one year and 85% in two years were recorded on plots on Highfield. This reduction is certainly due in part to the exposure of wireworms to their natural enemies and to the direct effect of disturbance by implements of cultivation. It has also frequently been suggested that the beetles prefer to oviposit on grassland or clover leys and to a lesser extent on cereal crops (Bryson, 1930; Rawlins, 1934; Balachowsky & Mesnil, 1935; Miles & Cohen, 1938). A laboratory experiment to test this point was accordingly carried out in June 1940.

Oviposition preferences

Twenty-five wooden boxes of 12 × 8 in. area and 8 in. deep were each filled with 28½ lb. of sieved Rothamsted allotment soil and the surface lightly pressed down to within ¼–½ in. of the top of the box. Five boxes each were sown on 13 May with winter wheat (Wilma), kale (thousand-headed), grass (mixture) and wild white clover. The remaining five boxes were left unsown. The wheat and the kale were sown in three rows about 3 in. apart across the width of the boxes, and the grass and clover were sown thickly over the whole area. The boxes were arranged in the form of a Latin square and clamped together on the floor of a well-ventilated, unheated glasshouse. A frame was erected around the boxes up to about 18 in. above their tops and, when the plants were well established, the frame was covered with muslin and all cracks at the sides and between boxes were sealed with surgical tape. The boxes were watered normally throughout the experiment and the unsown, wheat and kale boxes were kept weeded.

Between 8 and 13 June, 214 adults of *Agriotes obscurus* were introduced into the cage, eight or nine being placed in each box. *A. obscurus* was chosen because the previous year's observations showed that this species laid more readily in captivity than did *A. sputator*. The relative numbers of the sexes were not known as enough beetles could not be spared for dissection, and it was intended to make an examination of the survivors and the dead bodies at the end of the experiment. On 3 July the cage was opened, all crops were cut to ground level, and all beetles on the surface removed: two live beetles and seven dead ones were found.

The technique adopted for counting the eggs was as follows. Two cylindrical tins 12 in. high and 4½ in. diam. were each filled about one-third full of the soil to be examined (approx. 2½ lb.). The tins were then filled to within 1 in. of the top with a solution of magnesium sulphate (sp. gr. 1·12) and stirred for 5 min. The level of the solution was then raised almost to the edge of the tin and it was allowed to stand for 10 min. The level was again raised by allowing the solution to trickle in from an aspirator and the foam which formed on the top made it possible to raise the level nearly ¼ in. above the edge of the tin. This foam layer was carefully swept over with a straight edge into the enamel dish in which the tins were standing. The level was again raised and the liquid allowed to stand for a few more minutes. It was again swept over. The foam adhering to the sides of the tin and the straight edge was washed into the dish and the tins removed, the bottom also being washed. This mixture was then washed from the dish into a coarse strainer which retained all the roots and debris. The portion which passed through was filtered through a Buchner funnel using

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black filter paper on which the eggs could easily be seen. The foam was reduced by spraying it with alcohol. The residue in the strainer was then washed through with a strong jet of water and the wash was examined as above. This was repeated until no more eggs came through. The surface of the liquid in the tins was also examined after standing a further period and any eggs on it removed with a pipette. These were never more than one or two in number even when large numbers of eggs were found.

At first the liquid in the tin was stirred again for a second period of 2 min. and allowed to stand for 5 min. Invariably only a very small percentage (about 2% at the most) of the original number of eggs was found and this was neglected. Roots which remained at the bottom of the tin in the mud were also examined separately and, even with very high total numbers, only one egg was found in one lot and none in the others. This gives an indication of the efficiency of the method.

It was originally intended to examine only the top 2 in. because according to several workers (Roberts, 1919; Subklew, 1935; Miles & Cohen, 1939, and others) eggs are rarely laid below this depth. When the examination commenced, however, it was seen that the drying soil had retreated from the sides of the boxes leaving a wide crack all round extending some way down the box. It was therefore decided that at least the outer portion would have to be examined all the way down. In the first few boxes the top inch was examined as a whole, and then the centre and outside were examined separately in layers of about 2 in. In these lower layers no eggs were found in the central region, and it was decided to divide the top layer also into centre and outside. Here again, very few or no eggs were found in the centre and, ultimately, it was decided to examine the centre and outside (1 in. all round) of the top $2\frac{1}{2}$ in., and then only the outside portions of $2\frac{1}{2}$–5 and 5–$7\frac{1}{2}$ in. depths. The results are shown in Table 1.

TABLE 1. *Number of eggs laid in soil under various crops*

Kale	95	Wheat	202	Clover	89	Grass	345	Fallow	5
Grass	548	Clover	2	Fallow	0	Wheat	42	Kale	31
Wheat	83	Fallow	15	Kale	21	Clover	76	Grass	731
Fallow	0	Grass	205	Wheat	349	Kale	145	Clover	123
Clover	147	Kale	19	Grass	124	Fallow	0	Wheat	0

	Normal mean	Mean square roots
Grass	390·6	18·90
Wheat	155·2	9·70
Clover	87·4	8·55
Kale	62·2	7·26
Fallow	4·0	1·20
	S.E. ±2·33	

Only in three of the 23 boxes in which the top layer was examined in two parts had the central part any eggs; six in the centre of one grass box which had 456 around the edge, one in a wheat box with 39 around the edge, and one in the centre of another grass box with 208 round the edge. This seems to be strong presumptive evidence that the beetles went down the cracks to oviposit rather than burrow in the soil itself. In general most eggs were in the top layer and fewest in the bottom layer. There was a wide variation between the different replicates and it is difficult to understand why, for instance, in one wheat box there is nil and in another 349, and in one clover 2 and in another 147. Since the differences between the means were so great, the analysis was done on a square root transformation. The experiment was analysed as a Latin square.

The mean number of eggs laid in the grass boxes was significantly higher and in the unsown boxes significantly lower than any of the other three which do not differ significantly from one another. The low numbers found in the unsown boxes show that cracks alone are not sufficient inducement to oviposition, and the superior attraction of grass to clover suggests that full cover with shade and high humidity are not in themselves sufficient. An

interesting feature of the experiment was the disappearance of the beetles. Only two more were removed during the examination, making a total of 11 recovered altogether out of 214. When the cage was taken down no other beetles were found, although every care had been taken to make the cage beetle proof, and no beetles either dead or alive were noticed in the greenhouse after the boxes had been removed.

Movement of marked beetles

An oviposition preference such as had been demonstrated is hardly likely to be effective unless the beetles can travel comparatively long distances, sufficient to carry them over a normal-sized field. Although the beetles possess wings, most authors state that they have never seen the beetles in flight. Subklew (1935), however, has reported males of *A. obscurus* flying occasionally and Fryer (1941) has also observed *A. obscurus* in flight on several occasions.

A preliminary experiment to determine the distances that the beetles can travel was carried out in June 1940. 831 *A. sputator* adults were marked on the thorax with a spot of quick-drying red paint. On 5 June they were liberated in the centre of a strip of rather thin wheat at Rothamsted. The strip was 10 yd. wide separated from other cereal plots by a path 4 ft. wide on one side and by a fallow strip 11 yd. wide on the other. On 8 June bundles of grass, which had proved to be the most effective type of trap, were placed in lines across the width of the strip. The lines were 5 yd. apart and extended for 30 yd. each side of the centre. Four traps in each line were placed at equal intervals on the plot, and one trap in each line put just inside the cereal plots on either side making a total of 72 traps in all.

The traps were examined on 10, 12, 14 and 17 June for marked beetles. Some 200 were found dead near the point where they had been liberated, and it is probable that many others also died because they were in poor condition at the start of the experiment since it was nearing the end of the season. Thirteen beetles were recovered. Seven were caught in traps within a radius of 10 yd. of the liberation point. Two others were caught in the main plot in the 15 and 20 yd. radii respectively. Two more were found within 15 yd. radius on the nearer cereal plot and had crossed the narrow path. One was found within 20 yd. on the opposite cereal plot and must have crossed the wide fallow strip. The last one was found in the farthermost trap on the main plot, 30 yd. away. The weather throughout the period was warm and dry.

Relative numbers of *Agriotes obscurus* and *A. sputator* in different fields

In 1939 traps of lucerne and vetches were placed in Great Knott, a field ploughed from grass in that year, and in Pastures, a permanent arable field about 400 yd. away. The following numbers of beetles were caught:

	A. sputator	*A. obscurus*
Great Knott	778	45
Pastures	7	167

In May 1940 three recently ploughed fields and three permanent arable fields were examined to see if the same ratio of *A. sputator* to *A. obscurus* held. Preliminary tests that year showed that bundles of grass were superior to several other easily obtained plants as traps for the beetles. The bundles were about 1 ft. long and 3–4 in. diam. when tightly grasped. At first 10 traps were placed in each of the fields to be examined (with the exception of Great Knott where 24 traps for another experiment were used), but later the number

of traps was increased to 16 in those fields in which fewer beetles were caught. Table 2 shows the number of the two species caught between 25 and 31 May, the traps being examined every other day. Thus there was no evidence that there was any connexion between the species and whether the field was arable or recently ploughed, but there was evidence that the ratio of the two species did vary widely from field to field.

TABLE 2

	A. sputator	*A. obscurus*
Permanent arable fields		
Broadbalk (permanent wheat)	67	11
Hoosfield (permanent barley)	151	39
Long Hoos (3-course rotation, potatoes, barley, sugar beet)	49	139
Recently ploughed grassland		
Great Knott (ploughed spring, 1939)	1981	32
West Barnfield (ploughed autumn, 1940)	491	374
Sawyers (ploughed autumn, 1940)	254	35

DISCUSSION

Unfortunately, it has not been possible to continue with further experiments to clarify several points arising out of the investigation. While some sort of a preference has been demonstrated in the oviposition experiment, it may be that it is a food preference, and that having chosen the food they preferred, the beetles remained there to oviposit. A different result might have been obtained had there been no choice. The beetles will lay in bare soil, especially when it is loose and moist, and it may be that the watering of the fallow boxes caused a crust to form on the sieved soil though, as has been stated, cracks were present.

The movement experiment was on too small a scale to be of much value, though it does indicate that the beetles can travel short distances within a few days. If they are capable of travelling much greater distances one would not expect such a marked variation in the ratios of the two species in nearby fields unless each species is associated with certain conditions not yet determined. Miles & Cohen (1941) have shown that the beetles are much more active in cool moist weather, and suggest that movement in the spring is likely to be a movement in search of suitable temperature and moisture conditions.

With the slender evidence available so far it is impossible to decide to what extent the habits of the beetles are responsible for the smaller numbers of wireworms in arable than in grassland, and the need for further work along these lines is emphasized.

SUMMARY

In a laboratory experiment, *Agriotes obscurus* L. females laid significantly more eggs in grass, and significantly fewer eggs in a bare fallow, than in clover, wheat and kale which did not differ significantly from one another. Marked adults of *Agriotes sputator* L. were caught up to 30 yd. away from their liberation point, the maximum distance that traps were placed. The ratio of *A. obscurus* to *A. sputator* adults was shown to vary from 1 to 62 to nearly 3 to 1 in nearby fields.

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LABORATORY AND FIELD EXPERIMENTS ON THE CONTROL OF WIREWORMS

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1. Introduction

This paper is an account of experiments on the control of wireworms carried out at the Rothamsted Experimental Station in 1938–40. It opens with an account of a field experiment on the lines of those described by Ladell (1938). These experiments involved a great amount of work, usually with negative results, and it was decided to abandon them until laboratory tests had revealed new and more promising soil insecticides. As no suitable new substances were brought to light by laboratory tests, it was thought that an improvement in the application of the established fumigants would be worth investigating. This led to the experiments described in § 4.

2. Great Knott field experiment on the breaking up of grassland

In conjunction with the Rothamsted Farm, a large field experiment on the breaking up of grassland was planned for the autumn of 1938: beans, linseed, barley, oats, wheat and potatoes were the crops selected, the first two being considered resistant, and the remainder susceptible to wireworm damage. These crops were sown on $\frac{3}{4}$ acre plots each replicated four times. Each plot was subdivided into three: one section treated with naphthalene at the rate of 15 cwt./acre, the second with calcium sulphide at the rate of 350 lb./acre, and the other left untreated. Naphthalene is widely recommended as a control for wireworms, and Ladell (1938) obtained a 60% kill applying it at the rate of 10 cwt./acre. Calcium sulphide, especially in the form of gas lime or 'Blue Billy', was at one time frequently used as a soil sterilizer. The field selected for this experiment was Great Knott which had been put down to grass in 1928.

Ladell's (1938) experimental and sampling technique was adopted and the wireworms were separated by the Ladell flotation method (1936). Eleven preliminary soil samples (6 in. cubes) were taken at random over the field in July 1938 and gave a total of 16 wireworms or 1·45 (S.E. = ±0·34) wireworms per sample (253,000 per acre). In five of these samples, the lower soil level from 6 to 12 in. was also examined but only two wireworms were found, and it appeared that the majority were still in the top 6 in. Although this number of 1·45 per sample was lower than in previous experiments, it was considered high enough to reveal significant differences of the order required, due to the treatments. Accordingly, the plots were marked out and one sample taken from each of the 72 subplots between 11 and 24 Aug. The ground after the summer drought was hard and considerable difficulty was experienced in sampling. From 72 samples only 46 wireworms were obtained, the numbers ranging from 0 to 3 per sample. This is a mean of 0·64 (S.E. ±0·09) wireworms per sample or about 112,000 per acre, a figure usually considered below the danger level to crops. It was possible, however, that a downward movement of wireworms to below the 6 in. sampling level had taken place during the month after the preliminary sampling, though it seemed unlikely that two-thirds of the total population had moved down in such a short period.

The ground was so hard that it was impossible to plough until too late for the sowing of the winter beans and wheat. It was decided therefore to sow these in the spring with the other crops. The experimental area was ploughed between 1 and 13 Feb. 1939. The fumigants for each plot had been weighed out in four equal lots, and each lot was broadcast as evenly as possible over a quarter of the plot. (In Ladell's experiments the fumigants were placed in the furrows.) The calcium sulphide was diluted with three times its weight of dry sand to give it more bulk. In order that the fumigants should be exposed to the atmosphere as little as possible, only as many plots were treated at one time as could be ploughed in one day. The weather was favourable, scarcely any rain

falling in the period, but some days were very windy and some of the finely divided calcium sulphide must have been blown away. The land was rolled and harrowed twice during the next fortnight, and the barley, wheat, oats and beans were drilled on 3 and 4 Mar.

A second series of 72 samples was taken between 18 and 26 Apr. Neither the linseed nor the potatoes had then been drilled. The mean number of wireworms was then found to be 1·79 (S.E. ±0·20) per sample (312,000 per acre) ranging from 0 to 8. As this was similar to the figure obtained in July 1938 it suggests that some downward movement did take place between then and the August sampling, and another upward one before April 1939. Another possible explanation was that wireworms too small to be detected in August had grown sufficiently during the winter to be found in April, but this suggestion is ruled out by a comparison of the different size groups, an increase in numbers having occurred in each group as is shown by the following figures:

Size group in mm.	<0·5	0·6–0·9	1·0–1·3	1·4–1·6	1·7–2·0	>2·0
Aug. 1938	0	9	29	14	2	0
Apr. 1939	6	21	38	38	21	1

The effect of the treatments on the wireworms. There was no serious wireworm damage to the crop and no difference was observable on the different plots. Miles & Cohen (1938) stated that comparatively little damage is done by wireworms in the first year after ploughing grassland, as they continue to feed on the decaying turf. Neither of the treatments, however, showed any reduction in the numbers of wireworms as compared with the control plots. The mean numbers of wireworms per sample for each of the treatments was: naphthalene 1·79, calcium sulphide 1·87, and the control 1·71. The analysis of the experiment was as follows:

	Degrees of freedom	Sums of squares	Mean square	S.E.
Blocks	23	73·8750	3·2120	
Treatment	2	0·3333	0·1667	
Error	46	133·6667	2·9058	1·70

The only reason suggested for the failure of naphthalene in this experiment compared with Ladell's kill of 60% is that the application was made in February in the Great Knott experiment at a time when wireworms are not usually considered to be active, whereas the fumigants on High Field II were applied in April.

The effect of the treatments on the crop. The treatment did not appear to have any visible detrimental or favourable effect on the crop. A stand count of the cereals was taken in early May, and though the naphthalene plots showed slightly higher counts than the calcium sulphide and untreated plots on both the wheat and the oats, this difference was not significant. The only significant treatment effect was the reduction in yield of oat grain by both fumigants.

3. Laboratory tests on soil insecticides

The difficulties of carrying out reliable tests of insecticides on soil insects are well known. Some investigators have attempted to avoid these difficulties by exposing the insects to the substance to be tested in air (or sometimes water) instead of soil. It was usually found that the results in the two media were not comparable, and that frequently substances which were toxic in air were quite ineffective in the soil. The unnatural conditions for the test insect also lessen the value of such work. At the outset therefore, it was decided to perform experiments in soil and to try to eliminate sources of variability by standardizing the conditions as rigidly as possible.

The type of container in which such experiments are carried out is important. Tattersfield (1928) showed that a given concentration of naphthalene killed wireworms in a closed container, but in practice before that concentration was reached the wireworms were repelled, and if free to do so moved away. Thus, it was considered desirable to give the test insects a chance of escaping at least downwards from the region containing the insecticide, and also to be able to observe the extent of such movements. This was done by using a series of shallow cylindrical containers or units arranged vertically on top of one another each with a bottom of coarse wire mesh ($\frac{5}{16}$ in.), which was sufficiently open to permit the free movement of most insects and sufficiently close to retain soil. The units were made of galvanized iron and were slightly smaller in diameter at the bottom than at the top, so that the bottom of one fitted into the top of the next. They were about 8 in. diam. and 4 in. deep.

In devising a standard procedure the following factors had to be considered:

(1) *Temperature.* In order to carry out experiments at temperatures similar to those prevailing in the soil, drain pipes about 2 ft. long and slightly wider than the units were sunk vertically into the soil and the sets of units lowered into them by means of wire cradles. Owing to the variation of soil temperature at different times of the year this was not found to be satisfactory. Finally, an underground constant-temperature chamber working at 15° C. was constructed.

(2) *Type of soil.* The type of soil and its average particle size affect the distribution and sorption of the insecticide. While it was later intended to carry out experiments on several different types, in all the experiments described here, Rothamsted allotment soil sieved through $\frac{1}{4}$ in. mesh was used.

(3) *Soil moisture.* The percentage of water in the soil affects the diffusion and sorption of the insecticide and possibly has a direct effect on the insect. It is difficult to bring a large volume of soil (5–10 cwt. were usually handled at a time) to a given water content, and a trial and error method was employed. The standard selected was 18 parts by weight of water per 100 parts by weight of dry soil. At this moisture content the soil used was fairly easy to crumble but was sufficiently moist to hold together when squeezed firmly. If the soil after sieving was judged by feel to be much drier than this, water was added from a sprinkler can: if much wetter it was dried on trays.

Samples of the soil were dried for 48 hr. at about 40° C., and if the moisture content determined by this means was lower than 16% or higher than 20%, that series of experiments was rejected. After some practice it was usually possible to bring the soil to 17–19%. When a liquid insecticide was used the soil was ultimately saturated by it. It was still considered important to standardize the moisture content before the liquid was applied, as the amount of poison retained would depend on the amount of water previously present.

(4) *Soil compactness.* Like factors 2 and 3 with which it is intimately bound up, the degree of compactness by affecting pore space influences diffusion; also, where the soil is very compact the movement of the insects is impeded. The most convenient method of regulating the compactness was to weigh a given amount of soil into each unit and tamp it down lightly to a fixed mark. In the containers used, 7 lb. of soil pressed down to within 1 in. of the rim (i.e. in about 150 cu. in.) was found to give a fairly normal degree of compactness. The 7 lb. was poured into the container in two equal portions, and as far as possible the same pressure exerted on each.

(5) *Period of exposure.* Soil insecticides are allowed to act as long as they are present in the soil, and it was thought that very short period experiments would be too far removed from practice to be of much value. Six days was chosen as the minimum convenient period which would give results comparable with those in the field.

(6) *Test insects.* The present paper deals entirely with wireworms, although a few experiments were carried out with other insects. The wireworms were all of the genus *Agriotes* Esch., but may have belonged to any of the three common species *A. obscurus* L., *A. sputator* L. and *A. lineatus* L. They were obtained from various sources in Hertfordshire by advertising in the local press. Prior to experiments they were kept in soil in large flowerpots in an outdoor insectary. Potatoes or wheat grains were placed in the pots as food. The wireworms chosen for tests were all larger than 1·2 cm. and as nearly as possible of similar size. Individuals which were obviously about to moult or had recently moulted or were abnormal in any way were rejected.

General procedure adopted

Four units were commonly used together. The lowest unit of each set had a perforated zinc disk soldered to its bottom. The perforations were sufficiently small to prevent the escape of wireworms but not to impede drainage. When the lowest unit had been filled with the fixed weight of soil the

next unit was pressed down on the top of the soil and itself filled. In this way a better contact was assured between soil of adjacent units.

While the test insects and insecticides could be placed in any one or more of the units, in all the experiments dealt with here, both the wireworms and the substances to be tested were placed in the top unit. Twenty wireworms were placed in a layer 1½ in. below the surface of the soil. When a solid substance was to be tested it was ground to a fine powder if necessary and intimately mixed with the soil to be used in the top unit: when very small amounts of the substance were used it was diluted with talc to improve the distribution. When liquids were being tested three methods were used:

(1) Sludges and oily liquids like creosote were absorbed in sawdust and then mixed with the soil as solids.

(2) Small quantities of volatile substances were poured into a hole 2 in. deep made in the centre of the top unit.

(3) Some of the substances were used as aqueous solutions or emulsions. The total quantity of liquid added was always the same, an amount corresponding to 2 gal./sq. yd. Under the prescribed conditions this usually penetrated to the bottom of the third container. As emulsifying agents soft soap, turkey red oil (sulphonated castor oil) and sulphonated lorol were employed, whichever proved best for a particular substance being used regularly for that substance. To assist even distribution, the total amount of liquid required (360 c.c. for these units) was poured on as quickly as possible so that a pool formed on the surface of the top unit.

After 6 days the units were examined separately, the soil being sorted by hand and the distribution and condition of the wireworms noted.

Many of the insects were moribund at the end of the test, and all wireworms, except those certainly dead, were transferred to fresh soil in glass jars containing a piece of potato tuber, and examined at weekly intervals for 1 month. At the end of that time they were classified into the following four groups: (1) apparently normal in every way, (2) normal in appearance but rather sluggish in movement, (3) not or scarcely moving but still showing some signs of life when stimulated, and (4) dead. For working out percentage kills, groups 1 and 2 were considered alive and groups 3 and 4 dead.

At least one series of controls was carried out with every batch of tests. Where a solid was diluted with talc or a liquid absorbed in sawdust, an equal amount of talc or sawdust was added to the control series. Similarly, a solution of the emulsifying agent in water was added to the control series when the substance to be tested was applied as an emulsion. Mortality in the controls was unusual during the experimental period, but often one or two wireworms out of the twenty died during the month of examination afterwards.

The results of some preliminary tests with several miscellaneous substances are given in Tables 1 and 2. In order to facilitate comparison and to give the maximum amount of data in the minimum space the percentage kill obtained at various rates corresponding to cwt./acre are given in Table 1. (1 cwt./acre = 10·5 g./sq. yd. = 0·4 g./area of the units used.) In Table 2, which deals with liquids, the percentages are related to c.c./sq. yd., and in brackets under each figure is given the percentage of the original substances used where an emulsion was applied at the rate of 2 gal./sq. yd. The method of application is given after the name of the substance.

After these preliminary tests had been completed, it was intended to carry out five replicate tests using 20 wireworms in each at several different concentrations. At this stage, however, an entomogenous fungus* attack developed in the wireworm stocks and caused mortality in both the control and treated wireworms, and the results of these experiments were invalidated. Even in those tests which were satisfactory there was a great variation between replicates carried out at the same time and at different times.

In addition, the effect of dissolving naphthalene, chlor-naphthalene, and paradichlorobenzene in benzene forerunnings and carbon disulphide before emulsification was investigated (cf. Krauss, 1931; Miles & Cohen, 1939). Not only was the toxicity of the original

* The fungus was identified by the Imperial Institute of Mycology as an undescribed species of *Synglio-cladium*.

substance enhanced but the mixture was effective over a longer period. Detailed references to previous users of the insecticides are not given, as it is hoped to publish a bibliography of soil insecticides in the near future.

TABLE 1. *Percentage kill of wireworms by various substances*

Substance	Added to soil in amounts equivalent to cwt./acre											
	0·5	1·0	1·5	2	3	4	5	10	15	20	25	30
Ammonium persulphate (in sol.)	.	.	.	0	.	0	.	0	.	0	.	.
Barium sulphide	.	5	.	.	.	5	.	5	0	0	.	.
Creosote I (absorbed in sawdust) (boiling range 190–280° C.)	.	15	.	5	.	.	85	100	.	100	.	.
Creosote II (boiling range 220–363° C.)	.	35	.	20	.	.	75	90	.	100	.	.
Creosote III (boiling range 228–412° C.)	.	.	.	.	.	.	0	50	.	100	.	.
Mercuric chloride (in sol.)	0	0	.	0	5	.	.	.	.	.	.	.
Mercurous chloride	15	5	.	10	5	.	25	.	.	.	.	.
Naphthalene (flake)	.	.	.	25	.	.	35	50	42	40	80	100
Naphthalene (whizzed)	.	.	.	.	.	.	.	10	37	40	45	45
Paradichlorobenzene	.	15	.	15	40	60	100	.	.	.	.	.
Phenol	.	0	0	0	.	.	45	100	.	.	.	.
Phenol (in sol.)	.	0	5	15	.	.	65	95	.	.	.	.
Phenyl mercuric acetate	.	.	.	0	.	.	0	10	45	.	.	.

TABLE 2. *Percentage kill of wireworms by various liquids*

Substance	Added to soil in amounts equivalent to c.c./sq. yd.								
	23 (0·25)	47 (0·5)	70 (0·75)	94 (1)	187 (2)	281 (3)	374 (4)	562 (6)	749 (8)
Benzene forerunnings (emulsion)	.	40	.	50	52	.	88	84	94
Carbon disulphide (emulsion)	14	18	.	55	85	90	100	.	.
	31	38	.	61	94	100	97	.	.
Dichlorethylene (emulsion)	.	.	.	.	80	.	100	.	95
	.	5	.	5	45	.	80	95	.
ββ-Dichloro-ethyl-ether (emulsion)*	85	85	95	.	.	.	.	.	.
	95	85	100	100	.	.	.	.	.
Methyl allyl chloride (pure)†	.	25	.	40	75	70	80	.	.
	.	.	.	60	85	75	90	.	.

The figures in brackets give the percentage of the substance used when applied as an emulsion at the rate of 2 gal./sq. yd.

4. THE PREVENTION OF WIREWORM ATTACK ON CEREALS BY THE PLACING OF SOIL FUMIGANTS AND FERTILIZERS NEAR THE SEED

In an experiment to test the effect of fertilizers sown in contact with the seed, McMillan & Hanley (1936) noticed that wireworm damage to barley was much less severe where a mixed fertilizer had been drilled with the seed than where the fertilizer had been broadcast. Miles & Cohen (1938) recorded decreased wireworm injury to seedling wheat when superphosphate had been mixed with the seed. If this effect was due to the high concentration of the fertilizer repelling the wireworms from attacking the plant in its early, most susceptible stages, it might be possible to secure a similar result with soil fumigants which could be thus applied more economically than by mixing them with the whole area of soil. The application of these substances in direct contact with the seed would almost certainly

* See Lehman (1933), Campbell & Stone (1937). † See Briejèr (1938).

kill it, so that the substance would probably have to be applied 1 in. or so away from the seed as fertilizers have recently been applied in America. Miles & Cohen (1938) found that naphthalene in contact with wheat seed killed it. It is recognized that fertilizers themselves are frequently toxic when sown in contact with the seed, but McMillan & Hanley only found a slight delay in germination which was soon made up.

Exp. I. This was to test the protection of wheat seedlings by naphthalene: 24 porcelain pots 9 in. diam. and 10 in. high, of the type used in pot-culture work, were filled with 17 lb. of sieved Rothamsted allotment soil to a depth of 6 in. and 10 wireworms were placed on the surface of the soil in each of 12 of the pots. This corresponds to the high rate of infestation of almost 1,000,000 per acre. Nine of these pots were then filled up with 8½ lb. of soil for a further 3 in. and the remaining three were filled up with 8½ lb. of soil thoroughly mixed with 1·6 g. of powdered crystalline naphthalene. This corresponded to a rate of about 3 cwt./acre.

In three of the pots, two furrows 3 in. apart and 1 in. deep were made and 0·4 g. naphthalene (0·8 g./pot) spread in the bottom of each furrow. This corresponds to 3 cwt./acre applied to the central rectangular area 5 in. wide and 6 in. long. The total amount of naphthalene per pot is not therefore the same as that applied mixed with the top layer of soil of the first-mentioned pots. Ten wheat seeds were placed in contact with the naphthalene in each of these furrows and covered. Another three pots had three furrows each receiving 0·27 g. naphthalene (again giving a total of 0·8 g./pot) 3 in. apart and the 10 wheat seeds were sown in each of two similar furrows 1 in. deep between the naphthalene furrows. In the last three pots the wheat alone was sown as a control. In a second series of 12 pots, exactly the same routine was followed except that no wireworms were used. The pots were sown on 6 Oct. 1939, and were left in a large, well-ventilated glasshouse until April 1940 when they were taken outside. Counts of germination and plants surviving were made at intervals until harvest time, though no appreciable damage was noticed after mid-November when the young plants were well advanced. When the soil from three of the wireworm pots was examined in July 1940 only four wireworms were found.

TABLE 3. *Protective effect of naphthalene on wheat sown 6 Oct. 1939*

	Seeds germinated out of 20			Mean	No. of plants surviving 8 Nov. 39			Mean	No. of plants surviving 9 May 40			Mean
Wireworms present:												
No treatment	2	0	2	1·3	0	0	2	0·7	0	0	2	0·7
Naphthalene mixed with top 3 in. of soil	0	0	0	0	0	0	0	0	0	0	0	0
Naphthalene drilled with seed	2	0	0	0·7	2	0	0	0·7	2	0	0	0·7
Naphthalene drilled near seed	16	18	15	16·3	16	17	13	15·3	14	15	13	14·0
No wireworms:												
No treatment	16	19	15	16·7	16	19	15	16·7	15	18	12	15·0
Naphthalene mixed with top 3 in. of soil	0	0	0	0	0	0	0	0	0	0	0	0
Naphthalene drilled with seed	0	0	0	0	0	0	0	0	0	0	0	0
Naphthalene drilled near seed	20	17	17	18·0	20	17	17	18·0	19	17	17	17·7

Table 3 gives the results of the experiment which show that, while the naphthalene drilled near the seed effectively protected it from wireworm attack it had no deleterious effect on germination, as it had when broadcast or drilled with the seed. The poor germination in the no wireworms, no treatment series was probably due to old seed being used, and the later losses in the pots with no wireworms were due to a fungus.

Exp. II. In a similar experiment to test the effectiveness of superphosphate as a protectant, wheat was sown on 22 Nov. 1939. Forty-eight 12 in. flowerpots were loosely packed with sieved allotment soil and in one-half of them 10 wireworms were placed: 20 grains of Wilma winter wheat in two rows were sown in furrows 4 in. apart and 1 in. deep. For the purpose of the experiment the effective

area of the pot was taken as the central rectangle of $9\frac{1}{2} \times 7$ in. area. The superphosphate was then applied in the following ways at rates corresponding to 3 cwt./acre:

(1) Broadcast over the rectangular area and scratched in to about 1 in. deep (1·5 g./pot).
(2) Placed in the furrow with the seed (0·75 g./furrow).
(3) Placed in a similar furrow 1 in. away from each row and 1 in. deep (0·75 g./furrow).
(4) Placed in a similar furrow 2 in. away from each row and 1 in. deep (0·75 g./furrow).
(5) Placed in a similar furrow 1 in. away from each row and 2 in. deep (0·75 g./furrow).
(6) No treatment.

There were thus four replicates of each of the six treatments with and without wireworms. The pots were kept in a large, well-ventilated greenhouse until Apr. 1940, when they were taken outside. Regular counts of germination and surviving plants were made up to harvest in July 1940. Immediately after sowing, a cold spell started and germination was slow though almost complete by 12 Dec. While a few seeds in the wireworm pots were evidently attacked before germination, no damage was visible in those plants which had appeared above ground until the following spring. The figures in Table 4 show this period of activity. The numbers of surviving plants when maturity was reached are practically the same as those given for 9 May, and the final results are based on this record.

The tillering of the few plants left in pots severely attacked by the wireworms often brought the number of heads of wheat at harvest time to a similar number to that in the control pots where no or little tillering had occurred. Owing to the restricted space available in the pots it is considered that the number of plants surviving before they became too large for the pot is a better indication than the yield of grain of the actual results that might be expected in the field.

The figures in the column headed 9 May 40 in Table 4 were converted to percentages, and these were compared by using the inverse sine transformation, the most suitable means of comparing percentages varying over a wide range. The treatment totals for the converted data are given in the last column of Table 4, and differences of about 30 between them are significant. Thus the no-treatment and broadcast superphosphate are significantly different from all the others but not from each other. Superphosphate drilled 1 in. away and 2 in. deep is significantly different from all the others except the one drilled 2 in. away and 1 in. deep which is not significantly different from that drilled 1 in. away and 1 in. deep. They are all significantly different from the corresponding figures for no wireworms. There is thus evidence that superphosphate drilled near the seed is of some use in preventing wireworm attack either by increasing the resistance of the plants or by actual repellent effect on the wireworms.

Exp. III. This was carried out exactly as above and was commenced on 29 Nov. 1939. It showed that barium sulphide at 3 cwt./acre (which had given some evidence of a repellent action in insecticide tests) had no protective action when drilled with the seed or 1 in. from it. A mercurial seed dressing containing plant hormone was also quite ineffective in these experiments in preventing wireworm attack.

	Plants surviving on 25 Apr. 40							
	No wireworms			Mean	Wireworms			Mean
No treatment	20	16	19	18·3	7	1	7	5·0
Barium sulphide with seed	20	20	19	19·7	0	4	1	1·7
Barium sulphide 1 in. from seed	19	17	19	18·3*	0	3	12	5·0
Seed dressing	16	18	20	18·0	0	0	—	0

Exp. IV. In view of the promising results obtained in Exp. I, it was decided to perform a larger experiment in the spring. In order to economize the limited greenhouse space, rectangular wooden boxes of 12×8 in. area and 8 in. high were used. They had six drainage holes in the bottom covered with perforated zinc to prevent the escape of the wireworms. As before they were filled fairly loosely with sieved allotment soil. Barley was chosen instead of wheat, as barley is normally a spring-sown crop, and naphthalene and fertilizer were tested separately and together. The fertilizer used consisted of one part by weight of ammonium sulphate, one part by weight of muriate of potash and

TABLE 4. *Protective effect of superphosphate on wheat sown 22 Nov. 1939*

	No. of seeds germinated out of 20 by 12 Dec. 39					No. of plants 9 May 40				
No wireworms:					Mean					Mean
No treatment	19	20	20	19	19·5	19	18	16*	19	18·0
Superphosphate broadcast	18	19	18	20	18·8	19	18	19	15*	17·8
Superphosphate drilled with seed	20	19	19	17	18·8	20	20	20	17*	19·3
Superphosphate drilled 1 in. from seed and 1 in. deep	20	19	20	16	18·8	20	19	19	18	19·0
Superphosphate drilled 2 in. from seed and 1 in. deep	20	15	20	18	18·3	18	18	19	19	18·5
Superphosphate drilled 1 in. from seed and 2 in. deep	17	19	20	20	19·0	19	18	18	20	18·8

	No. of seeds germinated out of 20 by 12 Dec. 39					No. of plants out of 20 surviving on 16 Mar. 40					27 Mar. 40					9 May 40					Treatment totals of transformed data
Wireworms:					Mean					Mean					Mean					Mean	
No treatment	16	14	13	19	15·5	16	14	12	18	15·0	5	9	8	12	8·5	2	3	4	5	3·3	94·4
Superphosphate broadcast	15	18	14	17	16·0	15	18	15	16	16·0	9	6	10	9	8·5	2	3	6	6	4·3	107·6
Superphosphate drilled with seed	19	19	15	19	18·0	19	20	14	17	17·5	18	11	10	13	13·0	15	5	6	5	7·8	153·2
Superphosphate drilled 1 in. from seed and 1 in. deep	16	18	20	18	18·0	16	19	20	18	18·3	13	17	16	14	15·0	8	13	11	12	11·0	191·6
Superphosphate drilled 2 in. from seed and 1 in. deep	19	19	18	18	18·5	20	20	18	18	19·0	18	20	15	18	17·8	12	17	6	16	12·8	214·6
Superphosphate drilled 1 in. from seed and 2 in. deep.	18	19	20	20	19·3	18	20	20	20	19·5	16	18	18	18	17·5	12	15	16	14	14·3	231·0

Converted data analysis

	S.S.	D.F.	M.S.	S.F.
Total	5743·00	23	—	—
Treatment	3985·28	5	797·05	—
Error	1757·72	18	97·65	9·88

* A wireworm accidentally present in the soil used for the control pots was responsible for these low figures.

two parts by weight of superphosphate. It was applied at rates corresponding to 4 and 8 cwt./acre. The rather high rates were selected, as it has been found at Rothamsted that plants grown in pots require approximately double the amount of fertilizer than would be applied in the field. The naphthalene was applied at rates corresponding to 1, 2 and 4 cwt./acre, and when used with the fertilizer the latter was broadcast at the rate of 8 cwt./acre. Where the substances were broadcast they were spread as evenly as possible on the surface and then worked roughly into the top inch or so of soil. Where applied in rows they were used exactly as in Exps. I–III. The boxes were sown on 29 and 30 Mar. and, as before, kept in a large well-ventilated greenhouse. Counts were made up to the time of crop maturity. Unfortunately, the soil contained an unusually large number of wireworms, so that about a third of the control boxes contained one or two wireworms. The sterilizing of all the soil was not practicable, and it was hoped that during the sieving most of the few wireworms that were usually present in the allotment soil could be removed.

TABLE 5. *Effect of naphthalene and combined fertilizer on barley sown* 29 *and* 30 *Mar. in presence of wireworms. Numbers of plants out of* 20 *surviving on* 6 *May in three replicates of each treatment*

		A. Applied with fertilizer broadcast at 8 cwt./acre			B. No fertilizer		
I. NAPHTHALENE							
(i) Broadcast	1 cwt./acre	2	1	1	7	0	1
	2 ,,	4	1	4	10	1	1
	4 ,,	2	0	0	0	0	1*
(ii) 1 in. away 1 in. deep	1 ,,	13	3	10	4	6	5
	2 ,,	—	14	8	3	3	2
	4 ,,	6	5	13	9	9	0
(iii) 2 in. away 1 in. deep	1 ,,	7	8	7	3	5	3
	2 ,,	4	4	6	5	1	3
	4 ,,	10	0	0	1	1	1
	Mean = 4·02.						
II. FERTILIZER							
(i) Broadcast	4 cwt./acre	6	1	1			
	8 ,,	1	0	3			
(ii) Sown with seed	4 ,,	2	2	1			
	8 ,,	0	0	5*			
(iii) 1 in away 1 in. deep	4 ,,	6	3	2			
	8 ,,	10	0	4			
(iv) 2 in. away 1 in. deep	4 ,,	3	5	4			
	8 ,,	10	5	1			
(v) 1 in. away 2 in. deep	4 ,,	8	5	4			
	8 ,,	1	3	4			
	Mean = 3·33.						
III. CONTROL							
No treatment					5	0	3
					0	0	0
					4	0	1
					0	0	4
	Mean = 1·42.						

Analysis of variance

	S.S.	D.F.	M.S.	S.E.
Total	1055·68	94		
Treatment	543·43	28	19·41	
Error	512·25	66	7·76	2·79

* Denotes that the treatment affected germination in the corresponding control.

The results of this experiment are given in Table 5, expressed as the number of plants surviving out of 20 on 6 May in each box. The numbers of surviving plants in the control

boxes with no wireworms are not given, but where the treatment affected germination in these controls an asterisk is placed against the corresponding series in the table. There were three replicates of each treatment. Though the numbers are too low for the treatments to be considered satisfactory as a basis for control measures, some differences are significant. Thus, both the naphthalene and the fertilizer treatments are significantly better than the control. Naphthalene applied with the fertilizer is more effective than without. Naphthalene applied 1 in. away and 1 in. deep is significantly better than broadcast or 2 in. away and 1 in. deep in the fertilizer series, but is not or scarcely different in the no-fertilizer series. There are no significant differences between the various methods of application of the fertilizer in the second part of the experiment.

Summary

In a large-scale field experiment, neither naphthalene at 15 cwt./acre or calcium sulphide at 350 lb./acre applied broadcast in February and ploughed in, caused any decrease in the wireworm population. A technique for testing soil insecticides in the laboratory is described and brief notes given on results. In preliminary glasshouse experiments both naphthalene and superphosphate applied near the seed effectively reduced wireworm attack on wheat. A more elaborate experiment on barley did not give such good results.

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THE QUANTITATIVE INTERACTION OF SPRAY FLUID AND ACTIVE PRINCIPLE IN DETERMINING THE TOXICITY OF A PYRETHRUM PREPARATION TO THE ARGASID TICK, *ORNITHODORUS MOUBATA* MURRAY

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(With 3 Text-figures)

The tick, *Ornithodorus moubata*, is the alternative host of the spirochaetes of human relapsing fever in Central Africa. The disease is of some economic importance because, although the fatality rate may be low, sufferers are incapacitated for considerable periods. Both mature and immature ticks are potential carriers, and they frequent the habitations of man showing particular preference in their resting periods for cracks in walls and spaces in thatching. Fumigation is impracticable in most native houses, and control has often merely consisted in burning the infested hut, and rebuilding elsewhere. Contact insecticides seem to offer better promise, and this paper is incidental to research on the possibilities of this kind of application.

The tests with the tick were undertaken for the purpose of showing the effect of dilution with shell oil 24,210 on the toxicity of a stock 1·3% pyrethrins solution. A change in toxicity with dilution was naturally expected, and it was desired to see whether or not this change was relatively uniform throughout the mortality range, and also throughout a useful range of dilution.

Material and methods

First- and third-stage nymphs selected for experiment were fed approximately 24 hr. before treatment. Engorged ticks are more resistant than unfed ones, and it is easy to feed a batch for experiment all at the same time, and so ensure a uniform physiological condition throughout. The tick is most resistant to insecticides during a moult, but it is impracticable to culture groups to this condition for testing. Of a number fed at the same time, only a few will moult each day, and those few, at any given moment, will not have cuticles in an identical state of growth.

The reasons for leaving the engorged ticks 24 hr. before treatment are threefold: they have time to dry after their wetting with the coxal secretion (excess water excreted during feeding), they have time to settle down to the process of digestion, and a small percentage die through burst guts within some hours after a feed. The ticks were reared, and maintained if treated, in the dark at 30° C. and a relative humidity of approximately 50%.

Usually the number dead as a result of treatment could be safely counted within a week, but in some cases a longer time was necessary to decide which had survived and which had died. The majority of those which succumbed were dead by the second day after treatment. The symptoms of death include: presence of engorged blood in body cavity visible through integument; legs extended and no reaction to touching; tarsal joints flexed, other joints of legs relaxed.

The stock solution of insecticide used for most experiments was 'Pyremist L'*, a solution containing 1·3% weight in volume of pyrethrins in medium shell oil 24,210 (Potter, 1935). For these experiments the pyrethrins were not determined chemically, but one stock solution was used throughout, so that all the results are comparable. This was used neat and also variously diluted with shell oil 24,210 and odourless distillate.

* 'Pyremist L' is a proprietary preparation of Messrs Stafford Allen & Sons, London, who kindly supplied samples.

The specification of these two oils are as follows:

	24,210	Odourless distillate
Specific gravity (15–16° C.)	0·862	0·779
Initial boiling-point, °C.	325	198
Viscosity, seconds Redwood I	141	32
Flash point, °C.: Open	189	71
Closed	180	68
Unsulphonated residue %	99·2	99·0

The solution was atomized by means of an 'Aerograph' spray gun, type M.P. (nozzle no. 1) at a constant air pressure of 39 cm. of mercury (7½ lb./sq. in.). The spray was directed down a cylindrical glass tower, 44·5 cm. high and 11 cm. internal diameter, at the bottom of which were the subjects to be sprayed (cf. Potter, 1935). A weighed glass slide was always put on the sprayed area in order that the weight of insecticide per unit area could be ascertained after each spraying. There was a 1·7 cm. gap between the bottom of the tower and the base where the subjects were sprayed. Within the limits required the base received an even distribution of mist. This was seen both by a visual test in which coloured oil was sprayed on to filter paper, and a weighing test when cover-slips were placed so as to cover representative parts of the base.

Spraying the ticks directly as they crawled or rested in a Petri dish proved unsatisfactory for comparison of the effects of different concentrations and doses of insecticide. The spray fell on the dorsal surface, and until a large enough dose had accumulated to affect the lateral spiracles, no kill resulted. Above this critical dose, oil was as effective as undiluted insecticide, both giving a complete kill. There was no graded mortality. It was found that a tick with its spiracles or even one spiracle protected could be completely covered in oil on two successive days and yet survive to lead a normal life. Slight oiling of the two spiracles alone produced death by the following day.

Two alternative methods were employed: one for first-stage nymphs, in which they were put for 24 hr. on the smooth side of filter papers (no. 2 Whatman) which had received known doses and concentrations of 'Pyremist L' (Table 1); the other for third-stage nymphs, in which they were lightly fixed on their backs to a small strip of index card by means of a smear of rubber solution. They were then sprayed directly in this position, and released about 2 hr. afterwards on to clean filter paper (Table 2). By these two methods the insecticide gained access to the thin articular membranes of the legs which are relatively easily penetrated and a graded mortality was obtained depending both on dose and concentration. Potter (1935, 1938) was the first to use a film of contact insecticide on the substratum as a means of control for insect pests, and the modification of the method used here seems convenient for comparative purposes. Advantages of the filter-paper method for comparisons of the action of insecticides on ticks are as follows:

(1) The dose needed on filter paper is much easier to apply and measure accurately than the light doses required for direct spraying.

(2) In case of accident during the spraying, the only waste will be of filter paper, and not of livestock.

(3) The first-stage nymphs, the smallest active stage, can be used for experiments, and the trouble involved in rearing the later instars is avoided. Dosing would have to be immeasurably light for a direct spray on the first nymphs. Mortalities obtained in the filter-paper experiments and by direct spraying will be compared elsewhere. There was no mortality in control experiments.

Results

It is desirable to express administration of a contact insecticide in terms of dose of mixture and its concentration. In spraying experiments in particular the dosage is referred to as so many milligrams of mixture per square centimetre at so much percentage by weight of insecticide (Calloway & Musgrave, 1940; Potter, 1935, 1938). The dosage in these experiments was written in terms of weight of pyrethrins at each percentage concentration. Thus, where the usual terminology would describe a dose as '1 mg./sq. cm. of 1·3% pyrethrins', that used here would describe it as '0·013 mg./sq. cm. at 1·3% concentration'. For comparative purposes both terminologies are included in Tables 1 and 2. The advantage of graphing results in terms of weight of pyrethrins is that, where two or more concentrations

are plotted, the change in toxicity due to dilution of a certain weight of poison can be seen immediately by inspection. For the graphical presentation of results the 'probit' method of Bliss (1935*a*) was used.

TABLE 1. *The toxicity to first-stage nymphs of* Ornithodorus moubata *of pyrethrum-oil solution applied as a spray on filter paper*

Dose in mg./cm.²	Wt. of active principle mg. × 10³/cm.² at % concentrations				% mortality at concentrations			
	1·3	0·65	0·39	0·13	1·3	0·65	0·39	0·13
0·18	2·30	1·15	—	—	20	10	—	—
26	3·32	1·66	—	—	68 (45)	30	—	—
27	3·54	—	1·06	—	85	—	15	—
32	4·16	2·08 (40)	—	—	95	45	—	—
34	4·37	—	1·31	—	100	—	12 (100)	—
38	—	2·50	1·50	—	—	50	57·5 (40)	—
40	5·20	2·65	—	—	98 (40)	60	—	—
42	—	—	1·61	—	—	—	45	—
46	—	3·02	—	—	—	70	—	—
48	—	—	1·87	—	—	—	50 (40)	—
50	—	3·24	—	—	—	55	—	—
53	—	—	—	0·683	—	—	—	15
54	—	3·53	—	—	—	90	—	—
63	—	4·26	2·56	0·851	—	95	75 (40)	0
77	—	5·00	—	—	—	90	—	—
81	—	—	3·24	—	—	—	70	—
82	—	—	3·32	—	—	—	75	—
83	—	—	3·38	—	—	—	60	—
91	—	6·00	—	1·21	—	97·5 (40)	—	15
1·14	—	—	4·20	—	—	—	85	—
18	—	—	—	1·52	—	—	—	20
23	—	7·99	4·90	—	—	100	97·5 (40)	—
26	—	—	—	1·64	—	—	—	10
47	—	—	—	1·90	—	—	—	65*
57	—	—	6·07	—	—	—	100	—
65	—	—	—	2·15	—	—	—	10
97	—	—	—	2·57	—	—	—	55
2·10	—	—	—	2·67	—	—	—	35

The number of ticks was 20 per experiment, and if replications were done the total number of ticks used is stated in brackets in the mortality columns. * Oil alone begins to add to mortality at this dose. The stepped lines follow approximately equal weights of active principle at the different concentrations.

THE REGRESSION LINES

The equations of the regression lines (Table 3) and the tests were worked out following the method of Bliss (1935 *a*, *b*). The value of P is used as an indication of how well the points on the graph fit the calculated equation. The statistical method allows for sampling errors, care is taken to eliminate undue errors in technique of measuring doses, etc., and any discrepancy is usually put down to heterogeneity in the populations of animals used. Although care was taken to use ticks which were in a uniform state of nutrition and to avoid using ones of abnormal size, the heterogeneity evidently came about owing to changes in the resistance of the stock over the period of weeks during which the experiments lasted. The sprayings of the different concentrations were scattered as uniformly as possible over

this period. When $P < 0{\cdot}05$ the errors are taken as other than those of sampling. The trend in the equations nevertheless is uniform, viz. the coefficient of x rises and the constant a falls as the concentration of insecticide increases. The systems of points on the graphs seem sufficiently independent for certain conclusions to be drawn.

TABLE 2. *The toxicity to third-stage nymphs of* Ornithodorus moubata *of pyrethrum-oil solution applied as a direct spray*

Dosage in mg./cm.² at		Wt. of active principle in	% mortality at concentrations	
1·3 %	0·65 %	mg. × 10^4/cm.²	1·3 %	0·65 %
0·011	0·022	1·39	10	30
16	32	2·09	10	20
21	42	2·77	60	30 (20)
—	52	3·46	—	35 (20)
32	64	4·20	46·6 (30)	60 (20)
37	—	4·87	70	—
43	86	5·53	100	53 (49)
48	—	6·27	90	—
53	0·106	6·93	100	60
—	112	7·26	—	30
—	128*	8·32	—	60
—	138	9·00	—	55 (20)
—	148	9·74	—	60
—	158	10·40	—	85 (20)

The number of ticks was 10 per test except where otherwise stated in brackets in the mortality columns.
* At this dose oil alone begins to add to the mortality.

TABLE 3. *Regression lines worked out from the data in Tables 1 and 2*

Stage	% conc. insecticide	Equation $y = a + bx$	n	χ^2	P
1st nymphs on filter paper	1·30	$y = 0{\cdot}8666 + 9{\cdot}0884x$	3	2·8520	$> 0{\cdot}05$
	0·65	$y = 3{\cdot}4183 + 4{\cdot}4117x$	9	7·7608	$> 0{\cdot}05$
	0·39	$y = 3{\cdot}7647 + 4{\cdot}0006x$	9	28·1229	$< 0{\cdot}01$
	0·13	$y = 3{\cdot}8313 + 2{\cdot}1076x$	6	25·0243	$< 0{\cdot}01$
3rd nymphs sprayed directly	1·30	$y = 2{\cdot}7383 + 4{\cdot}2551x$	4	13·0162	$> 0{\cdot}01$
	0·65	$y = 3{\cdot}9175 + 1{\cdot}5391x$	10	11·4183	$> 0{\cdot}05$

n = the number of degrees of freedom of the data. χ^2 = a measurement of how far the observed points vary significantly in position from the expected points on the regression line. P = the probability that the scatter of the particular number of experimental observations about the fitted line has occurred purely by chance.

TABLE 3A. *The significance of the differences of slope of the regression lines*

Experiment	% conc. of pyrethrins	Regression coefficient $= b$	Variance of b	Comparison	t	P
On filter-paper	1·30	9·0884	1·8363	1·3 – 0·65	3·25	$0{\cdot}01 > P > 0{\cdot}001$
	0·65	4·4177	0·2230	1·3 – 0·39	3·40	$0{\cdot}01 > P > 0{\cdot}001$
	0·39	4·4006	0·3994	0·65–0·39	0·53	0·6
	0·13	2·1076	1·4229	0·65–0·13	1·80	$0{\cdot}1 > P > 0{\cdot}05$
Direct spray	1·30	4·2551	2·6476	1·3 – 0·65	1·61	$0{\cdot}2 > P > 0{\cdot}1$
	0·65	1·5391	0·1749			

Table 3A is a summary of the significance of the differences in slope of the regression lines. The regression coefficients are taken from Table 3. The variance of b is calculated

from a formula of Bliss (1935*a*). For two lines the value of t is the difference of the regression coefficients divided by the square root of the sum of their variances. P is a measure of the probability that two regression lines have the same slope: if $P < 0{\cdot}05$, the two slopes compared may be taken as significantly different. The 1·3 % line for filter-paper is significantly different from the other filter-paper lines. The comparison of the 0·65 and 0·13 % lines is a border-line case, and the data for the direct-spray experiments allow of no differentiation of slope between 1·3 and 0·65 % lines. This may be explained partly by the heterogeneity of some of the lines (Table 3). The important points are the definite differences between the 1·3 and 0·65 % lines, both homogeneous, for the experiments on filter-paper, and the obvious correlation between slope of the regression line and concentration of insecticide.

TABLE 4. *Regression lines for the toxicity to first-stage nymphs of* Ornithodorus moubata *of a pyrethrum-oil solution applied to filter-paper. The oil was* 80 % *shell oil* 24,210, 20 % '*odourless distillate*'

% conc. of insecticide in pyrethrins	Regression equation $y=a+bx$	n	χ^2	P
1·04	$y=3{\cdot}5656+5{\cdot}0022x$	4	11·0013	$>0{\cdot}02$
0·52	(i) $y=3{\cdot}8528+1{\cdot}0932x$			
	(ii) $y=2{\cdot}5967+6{\cdot}3254x$	7	26·6593	$<0{\cdot}01$

TABLE 4A. *The data on which the regression lines in Table* 4 *were calculated*

Conc. of insecticide % pyrethrins	Dosage in mg./cm.²	Wt. of pyrethrins mg. × 10³/cm.²	% mortality	Conc. of insecticide % pyrethrins	Dosage in mg./cm.²	Wt. of pyrethrins mg. × 10³/cm.²	% mortality
1·04	0·093	0·965	5	0·52	0·093	0·485	10
	139	1·444	20 (15)		139	725	15
	173	1·780	55		187	970	10
	202	2·100	50		233	1·210	10
	279	2·900	100		325	1·700	23·3 (60)
	295	3·060	65		340	1·770	25
	310	3·240	90		371	1·940	10
					388	2·020	10
					435	2·260	55
					449	2·340	60 (35)
					480	2·500	70
					559	2·900	55
					700	3·630	80
					820	4·290	100
					1·080	5·650	100

The number of ticks treated at each dosage was 20 except where otherwise stated in brackets in the mortality column.

OTHER INSECTICIDES, MEDIA AND SUBJECTS

Two solutions of pyrethrins, 1·04 and 0·52 %, were tested in a medium of 8 parts shell oil 24,210 and 2 parts shell odourless distillate, a lighter and more volatile oil. There was no great difference in the slope of the regression lines at these two concentrations (Fig. 3), though the 0·52 % line had a flatter segment in the lower dosages. The lower concentration proved somewhat less toxic, weight for weight of active principle as shown in Table 4.

The partial replacement of shell oil 24,210 by odourless distillate in the mixture increases its toxicity considerably.

Calloway & Musgrave (1940) sprayed eggs of the bed bug, *Cimex lectularius*, with lethane 384, which is a mixture of equal parts of an organic thiocyanate and odourless distillate, and with a solution of pyrethrins in shell oil 24,210, two concentrations of each. They plotted their data in the usual manner using wt. of deposit/sq. cm. as the measure of dose. For the purposes of the present comparison the equations of the lower concentrations of these two insecticides have been transformed to make the doses of active principle indicated fall in line on a graph with corresponding doses at the higher concentration. The effect on the regression line is a movement to the left to the extent of the logarithm of the ratio of the higher to the lower concentration (Table 5).

TABLE 5. *Regression equations from Calloway & Musgrave* (1940) *showing the toxicity of lethane* 384 *and pyrethrum to the eggs of* Cimex lectularius. *The equations representing the lower concentrations have been altered in column* 3 *to make the weights of poison administered equivalent to those at the higher concentrations*

Insecticide	Equation	Transformed equation
6% lethane in odourless distillate	$y=4{\cdot}93x-0{\cdot}08$*	
4% lethane in odourless distillate	$y=4{\cdot}38x-0{\cdot}85$	$y=4{\cdot}38x-0{\cdot}08$
0·4% pyrethrins in shell oil 24,210	$y=1{\cdot}87x+2{\cdot}38$*	
0·1% pyrethrins in shell oil 24,210	$y=2{\cdot}37x+1{\cdot}15$	$y=2{\cdot}37x+2{\cdot}59$

* Heterogeneous (Calloway & Musgrave, 1940).

Comparing the equations of the higher concentrations with the transformed equations of the lower concentrations, there is not a marked difference in the respective toxicities (Table 5). 4% 'Lethane 384' is somewhat less toxic than 6% weight for weight of active principle, and 0·1% pyrethrins somewhat more toxic than 0·4% on the same basis. There are no large differences in slope such as are seen in the lines for pyrethrin solutions in shell oil 24,210 acting on nymphal ticks (Fig. 1).

DISCUSSION

(1) *Pyrethrins in shell oil* 24,210

Figs. 1 and 2 show that there is a crossing of the regression lines for the different concentrations of pyrethrins in both the filter-paper and the direct-spray experiments. The higher the concentration the steeper is the regression line, and the lower the point where it cuts the ordinate on the zero abscissa. This indicates that the lower weights of pyrethrins are more toxic to the tick if diluted, while the higher weights are more toxic if they are more concentrated. The weight of pyrethrum indicated at the crossing-point of two given lines is equally toxic at both concentrations. Thus in these experiments, when the pyrethrum solution was diluted and tested against the tick, there was not a uniform change from the original toxicity, but an increase in the lower part of the mortality range and a decrease in the upper part. The toxicity of a dose evidently depends on both its concentration and its bulk, or its inherent toxicity and its power of reaching the vulnerable parts of the tick. Now it is generally understood that diluting a poison makes it less toxic. With weights of pyrethrum at the lower end of the experimental range, the loss in toxicity due to dilution from, for example, 1·3 to 0·65%, is more than counterbalanced by the gain in toxicity

due to the increased contact with the tick caused by the extra bulk of the diluent. With weights of pyrethrum at the upper end of the experimental range, dilution causes a loss in toxicity notwithstanding the increase in bulk.

Shell oil 24,210 spreads fairly readily over the sculptured parts of the tick and over the articular membranes. No spreading takes place on the pale sclerites of the legs. On a clean glass slide it was seen that over a large range (0–90% area) the area covered by sprayed droplets was in linear relation with the weight of the deposit. (Camera lucida drawings were measured with squared paper.) The vulnerable parts of the tick's cuticle might receive oil in a similar fashion. Thus, doubling a light dose of oil would double the

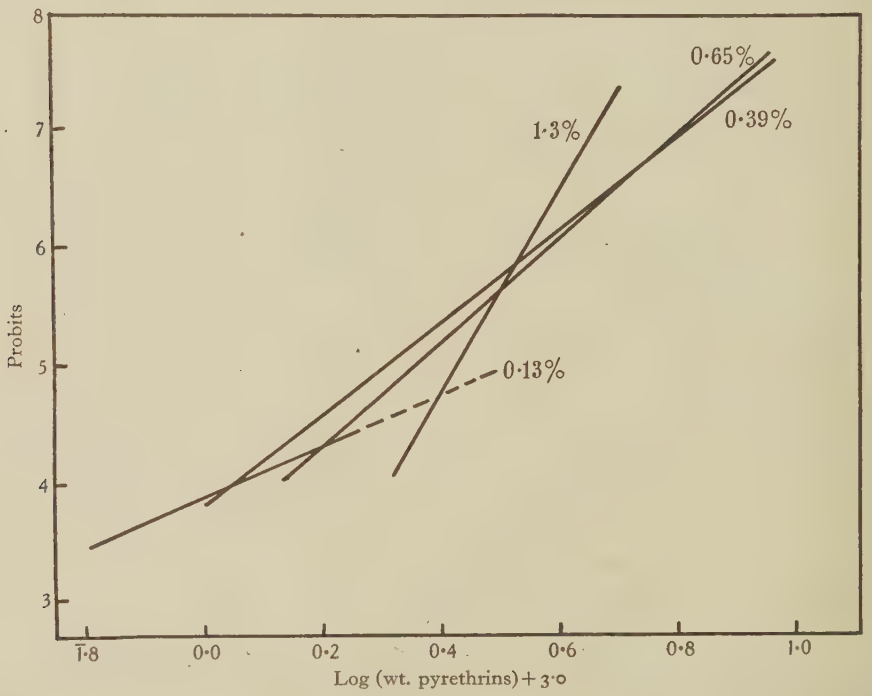

Fig. 1. Regression lines for denoted concentrations of pyrethrins in shell oil 24,210 used as a film on filter-paper against first nymphs of *Ornithodorus moubata* (Tables 1, 3). Each line covers the range of dose used for the concentration. The broken section on the 0·13% line represents the beginning of the region where oil alone is effective.

area of the cuticle covered, while doubling a heavier dose would produce only a relatively small increase in area. Finally, doubling a dose which gave complete cover would produce no increase in the area covered. Wilcoxon & Hartzell (1931) showed how increase in spreading ability alone adds to toxicity of a 0·1% solution of nicotine to *Aphis rumicis*.

Doses in the experimental range higher than the point at which all the vulnerable parts are covered would be dependent purely on increasing weight of insecticide for further increase in toxicity, with its possible entry into the spiracles. From evidence of spraying experiments with oil it was found that two of the regression lines extend into the range where oil alone begins to contribute to mortality by entering the spiracles: the 0·13% line

for first nymphs at the 1·5 mg./cm.^{2} point, and the 0·65 % line for third nymphs at the 0·12 mg./cm.^{2} point (Figs. 1, 2). It seems probable that a pyrethrum-oil mixture would be effective by way of the spiracles before these points, since entry of oil into one spiracle is not sufficient to cause death. On the other lines '100 % mortality' was reached before these points. The regression lines do not show any definite breaks which might indicate a change in the mode of entry of the poison, though if the change in slope were slight, experimental error would mask it. Except for the 0·13 % concentration applied to filter-paper, it is not thought likely (in data for pyrethrum where χ^2 is greater than would be expected for $P=0{\cdot}05$) that this discrepancy is accounted for by a change in physiological

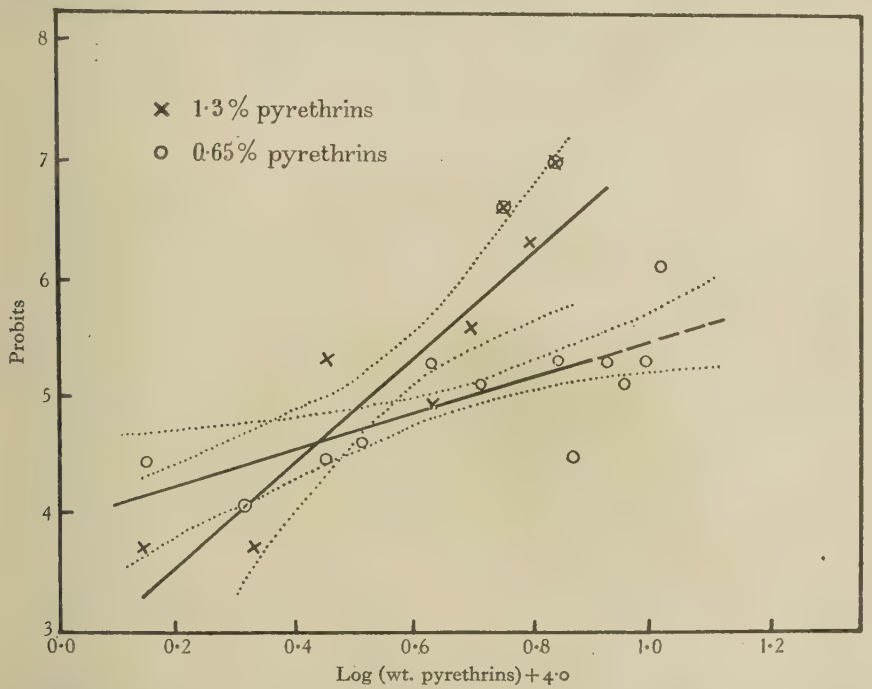

Fig. 2. Regression lines for 1·3 and 0·65 % pyrethrins in shell oil 24,210 used as a direct spray against third nymphs of *Ornithodorus moubata* (Tables 2, 3). The broken section on the 0·65 % line represents the beginning of the region where oil alone adds to the total mortality, and the dotted curves show the limits within which the lines have been determined by the data. Ringed crosses indicate '100 % mortality' with 1·3 % pyrethrins.

action within the range. More probably it is due to some heterogeneity in the ticks causing a few widely placed points on otherwise reasonably straight lines. Only the 0·13 % line goes far enough into the region where oil alone is effective for mortality to be increased on this account.

Stated briefly, bulk of insecticide seems the more important factor contributing to toxicity in the lower dosages, and concentration of active principle in the higher dosages. Beyond a certain optimum dose, extra bulk produces less and less relative increase in availability until the spiracles begin to be flooded, while inherent toxicity increases constantly with dose and concentration (Clark, 1933; O'Kane *et al.* 1930; Shepard, 1939).

(2) *Other insecticides, media and subjects*

As already noted, only slight differences of slope occur between lines representing dosage-mortality data for 'Lethane 384' in odourless distillate and pyrethrum in shell oil 24,210 against eggs of *Cimex lectularius* (Table 5). If bulk of insecticide contributes to toxicity here, it must act cumulatively side by side with the active principle on a more or less compensatory basis over the whole range explored. The lack of advantage of dilution at the lower doses is probably due to the already adequate covering of the vulnerable parts by those doses. Similar results would be expected with an insect more resistant to, or more easily covered by, Pyremist 'L' than is *Ornithodorus*.

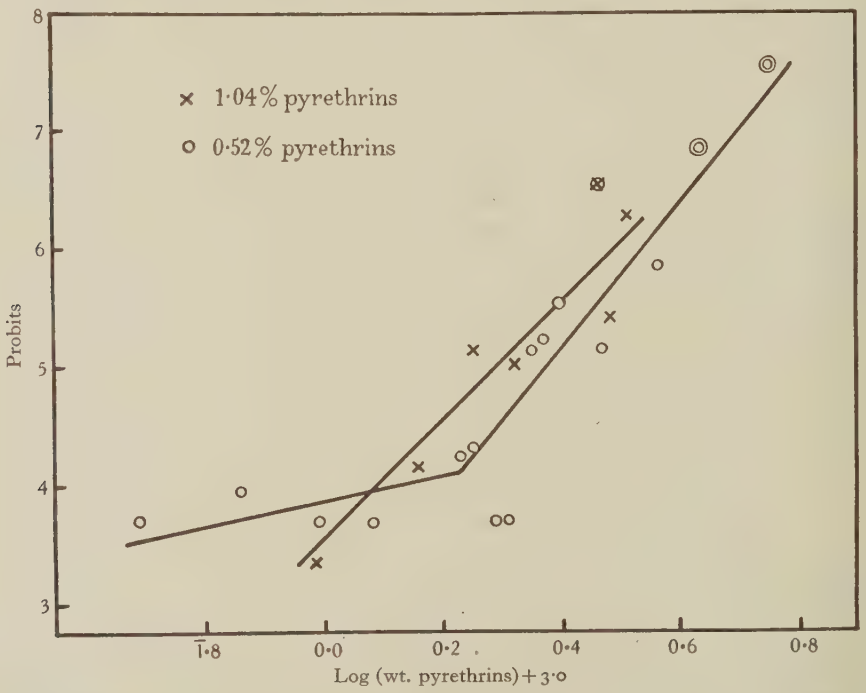

Fig. 3. Regression lines for denoted concentrations of pyrethrins in a mixture of 8 parts shell oil 24,210, 2 parts shell odourless distillate used as a film on filter-paper against first nymphs of *Ornithodorus moubata* (Tables 4, 4A). Ringed points represent '100% mortality' points.

It is noteworthy in this connexion that the addition of a proportion of odourless distillate to Pyremist 'L' gives nearly parallel regression lines for 1·04 and 0·52% pyrethrins used as a substratum spray against first-stage nymphs of the tick (Fig. 3). There is a well-defined flatter segment in the 0·52% line in the region of the lower dosages which need not be considered in detail here. Shepard (1939) mentions that such segments are often met with, and cites a fumigant acting on *Tribolium confusum*. Bartlett (1936) indicates that such segments may sometimes be due to a natural mortality of 5% or so. The controls in these experiments showed no natural mortality. It can easily be seen that a drop of shell oil 24,210 thinned with 20% odourless distillate spreads over the tick's cuticle much more

rapidly than unthinned oil. This explains why, in the region of the lower dosages, there is less increase of availability (and toxicity) with bulk in these solutions than when unthinned oil is used.

Among the many factors which decide whether or not a dose will kill an insect are the total amount of poison entering through the cuticle, the rate of its penetration, and the rate at which the tissues and excretory organs can render it harmless. In the instances of increase of toxicity with dilution mentioned above, the factor which is increased is the rate of entry due to the larger contact area between cuticle and spray, again notwithstanding the lower concentration. At doses in excess of that required to cover the cuticle a high concentration will be more toxic because concentration is the factor now controlling the rate of penetration. At any concentration, doses above that required to cover the cuticle increase the likelihood of death by virtue of the increase in the amount of poison which may eventually be absorbed.

(3) *Conclusions*

The relationships between regression lines representing different concentrations of pyrethrins acting on the tick (and possibly other insecticides acting on other insects) seem to be affected by: (*a*) the inherent toxicity and concentration of the active principle, and (*b*) the availability of the active principle to the tick by virtue of bulk or spreading capacity of the carrier.

If the insecticide shows a marked toxicity before it completely covers the cuticle, diluting it will make it more effective in the lower dosages but less so in the higher dosages. In other words, the regression lines for two concentrations will cross if there is considerable overlapping of the dose ranges of 5–95 % mortality and 5–95 % covering of the vulnerable parts of the cuticles of the test insects.

Summary

An extension of Potter's (1938) toxic film method of control of warehouse pests is described as a means for testing pyrethrum against ticks, *Ornithodorus moubata*. In spite of heterogeneity of some of the results detected by statistical methods, it is thought that the data allow the conclusion that the toxicity of the pyrethrum-oil spray to the tick depends on a balance between the inherent toxicity of the active principle and the bulk or availability of the dose. Diluting such a spray mixture will make it more effective against the tick in the lower dosages but less so in the higher dosages.

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THE USE OF TOXIC POLYNITRO DERIVATIVES IN PEST CONTROL

I. THE ESTIMATION OF DINITRO-*O*-CRESOL AND DINITRO-*O*-*CYCLO*HEXYLPHENOL

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3:5-Dinitro-*ortho*-cresol (D.N.C.) and stock emulsions of this substance in petroleum oil are available commercially and winter washes based on D.N.C.-petroleum are becoming increasingly popular with the fruit grower. These washes can replace the separate application of tar and of petroleum-oil emulsions, while the risk of bud injury which attends the use of combined tar-petroleum washes is avoided (Kearns & Martin, 1941). In view of the increasing importance of D.N.C. the chemistry of this substance becomes of interest and further, suitable methods of analysis, both for the compound itself and as a constituent of oil emulsions, are urgently required. In this paper a number of molecular compounds and derivatives of D.N.C., and two simple methods of analysis are described. The application of the methods to the estimation of D.N.C. in compounded products will be communicated at a later date.

Historical

(i) *Use as insecticides.* The superior insecticidal properties of dinitro-*o*-cresol and its salts over dinitrophenol were recognized in 1892 when the Farbenfabriken vorm. Friedr. Bayer A.G. marketed the potassium salt under the name 'Antinonnin' (Cooper & Nuttall, 1915) for use against the nun moth (*Lymantria monacha* L.). Jackson & Lefroy (1917) found that the ammonium and potassium salts of both 3:5-dinitro-*o*- and 3:5-dinitro-*para*-cresols were good stomach poisons against the house-fly and reported the *ortho*- to be much more toxic than the *para*-cresylates. Similar results were obtained by Hargreaves (1924) who compared the efficiencies of several nitrophenols as stomach poisons against the larvae of *Pieris rapae* L. and *Diacrisia lubricipeda* L. The relative toxicities of many nitrophenols as contact poisons were investigated by Tattersfield *et al.* (1925), who found that to the eggs of *Aphis rumicis* L. and *Selenia tetralunaria* Hufn., 3:5-dinitro-*o*-cresol was the most toxic substance examined. 2:4-Dinitrophenol and 3:5-dinitro-*o*-cresol were among the most promising of a wide range of organic substances tested by McAllister & Van Leeuwen (1930) against newly hatched codling moth larvae. Smith *et al.* (1938), also working with codling moth larvae, reported 3:5-dinitro-*o*-cresol to be one of the two most toxic of over 200 organic substances investigated.

The ovicidal efficacy of D.N.C. oils and salts has been demonstrated by a number of workers both in the laboratory and in the field (Gimingham *et al.* 1926; Gimingham & Tattersfield, 1927, 1928–9; Hey, 1938; Kearns & Martin, 1939; Shaw & Steer, 1939; Hough, 1939; Bushland, 1940; Hardy, 1940).

In America, following the discovery by Kagy & Richardson (1936) and Dutton (1936) that dinitro-*o*-*cyclo*hexylphenol (D.N.O.C.H.P.) incorporated in dormant spray washes was very effective in the control of the San José scale (*Aspidiotus perniciosus* Comst.), the rosy apple aphis (*Anuraphis roseus* (Baker)), and the black cherry aphis (*Myzus cerasi* (F.)), many studies have been made with this compound (for literature see Boyce *et al.* 1939 and Kagy, 1941). The consistently successful results obtained led Kagy (1941) to examine the insecticidal properties of other compounds of this type. 2:4-Dinitro-6-phenylphenol in the form of its calcium salt was not particularly successful as a stomach poison against the corn earworm (*Heliothis armigera* H.C.) (Kagy, 1936). In a later investigation of the relative toxicities to silkworms of various 2:4-dinitro-6-alkyl and *cyclo*alkyl phenols Kagy found the *n*-hexyl and *n*-heptyl compounds to be the most toxic substances, toxicity progressively

increasing with increase in the length of the alkyl chain up to six or seven carbon atoms. All these non-*aryl* derivatives were more toxic to silkworms than acid-lead arsenate but less toxic than rotenone.

(ii) *Analysis.* Although pure D.N.C. and related compounds can be analysed by the standard methods of organic chemistry, no simple and reliable routine method for the estimation of these substances has been reported. Fischer (1938) suggested three methods for the estimation of D.N.C. in plant-protection materials. In the gravimetric determination, a weighed amount of the sample was treated in 1% NaOH to remove basic and neutral constituents. The ether extract from this solution after acidification was weighed directly as D.N.C., a procedure which suffers the disadvantage that other phenols are automatically extracted with the cresol. His volumetric method involved a modification of the standard procedure for the estimation of nitro groups, namely, their reduction by boiling with titanous chloride in hydrochloric acid solution in an atmosphere of carbon dioxide, the excess of reducing agent then being titrated with a standard solution of ferric alum. It is necessary to perform a concurrent blank under identical conditions. The colorimetric determination described by Fischer depends on the production of an orange-red colour with aqueous potassium cyanide in presence of alcohol. The colour takes time to develop and conditions must be carefully controlled. The method, by which 0·1–1·5 mg. of D.N.C. can be estimated, although of micro-analytical use is rendered unsuitable for bulk samples on account of the large factor involved in working out the result.

An alternative colorimetric method used by Fischer for the determination of D.N.C. was applied to the estimation of dinitro-*ortho-cyclo*hexylphenol by Boyce *et al.* (1939). In this method the compound is converted into its sodium salt and the colour of this solution compared with that of a suitably prepared standard. The colour of dinitro*cyclo*hexylphenol in alkaline solution ranges from reddish orange to pale yellow so that the standard and unknown solutions must be of similar strength. At any particular concentration the colour is not changed within a *p*H range of 11–13, and the solutions for analysis are therefore adjusted within this range.

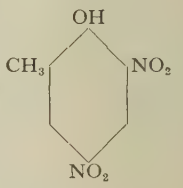
The standard method for the estimation of phenols in which the substance is brominated with standard bromide-bromate solution in presence of hydrochloric acid has been applied to 2:4-dinitrophenol by the Association of Official Agricultural Chemists (1937, 1939). In this substance, the nitro groups as well as the hydroxyl group direct substituents into the 6-position, and as would be expected, bromination occurs readily. In the molecule of D.N.C. however, the 6-position is occupied by a methyl group so that nuclear bromination is not likely to occur. Thus, it was found that D.N.C. does not react with bromine in glacial acetic acid solution in presence of a 'halogen carrier'. Methods of estimation involving bromination must therefore fail with D.N.C.

The quantitative determination of the nitro group in organic compounds involving reduction by stannous chloride has been modified by many workers since it was first introduced by Limpricht (1878), but in spite of many attempts to eliminate apparent sources of error, generally satisfactory results have not been obtained. Hinkel *et al.* (1939) discuss in detail the errors affecting the estimation, and working with purified reagents and under controlled conditions obtained concordant results for a large variety of nitro compounds including D.N.C. Like the volumetric method described by Fischer, it is essentially an estimation of the nitro group. Following a long series of similar experiments in this laboratory in which titanous sulphate was used as the reducing agent, it was decided that such a method was too involved for routine use and simpler methods were sought. For this purpose the general physical and chemical properties of the compound were examined and a number of new derivatives both of D.N.C. and D.N.O.C.H.P. were prepared.

General properties of D.N.C.

Dinitro-*o*-cresol, or more correctly 2:4-dinitro-6-methylphenol, when pure is a yellow, almost odourless solid of m.p. 86° C. It is an acidic substance forming well-defined salts with inorganic bases, the NH_4, Na, K, Ca and Ba salts being soluble.

The commercial substance may be obtained in the form of brownish granules but is more frequently met with as a yellow powder to which 10% or more of water has been added to reduce fire hazard.



Dinitro-*o*-cresol

Solubility in organic solvents

	g. D.N.C. dissolved by 100 g. solvent at 15° C.		g. D.N.C. dissolved by 100 g. solvent at 15° C.
40·60 petroleum ether	0·51	Glacial acetic acid	23·45
Carbon tetrachloride	2·40	Benzene	37·15
Ethyl alcohol	4·30	Chloroform	37·20
Methyl alcohol	7·33	Acetone	100·60
Ether	9·12		

Tests for D.N.C.

(1) Soluble in 7813 parts of water at 15° C., in 2068 parts at 100° C. The yellow colour of the aqueous solution is discharged by hydrochloric acid.

(2) Aqueous ferric chloride added to a solution of D.N.C. in alcohol gives a colour ranging from yellow to deep red. At a concentration of 0·5 % D.N.C. it is orange red.

(3) Warmed with alkaline sodium hypochlorite solution yields chloropicrin (b.p. 112° C.), easily recognized by its characteristic acrid odour.

(4) Formation of addition compounds (Table 1).

(5) Acetate, m.p. 96° C.; benzoate, m.p. 132° C.; methyl ether, m.p. 72° C.

Molecular compounds

A preliminary investigation having shown that the solubilities of the salts of D.N.C. with heavy metals were too high to permit their use in a gravimetric estimation, the question of molecular compound formation was considered. The molecule of D.N.C. is reasonably symmetrical, and since it contains two strong electron-attracting groups would be expected to form crystalline molecular compounds (e.g. Bennett & Wain, 1936). This was found to be the case, compound formation occurring readily with a number of amines, heterocyclic bases, hydrocarbons and phenols* (Table 1). Absolute alcohol was found to be a suitable solvent in the preparation of these complexes and was used throughout.

The two components were mixed, usually in equimolecular proportions but sometimes with an excess of one, in warm alcoholic solution and the compound which crystallized was filtered off. This was not usually recrystallized but analysed directly (Table 1).

Estimation of dinitro-*o*-cresol

(1) *Volumetric method*

The feebly acidic properties of phenol are markedly increased by the substitution into the nucleus of nitro-groups (e.g. Treadwell & Schwarzenbach, 1928). Thus, although solutions of phenol itself do not liberate iodine from a solution of iodide-iodate, the concentration of hydrogen ions in a solution of D.N.C.† is sufficiently high to bring about the reaction

$$5I' + IO_3' + 6H^+ \rightarrow 3H_2O + I_2.$$

This property has been utilized in the volumetric estimation of D.N.C.

* The products are all referred to here as 'molecular compounds'. Where the second component is basic, the constitution is more probably that of a salt.

† Similarly, D.N.C. liberates carbon dioxide from a solution of sodium carbonate whereas phenol itself does not.

TABLE 1. *Molecular compounds*

First component	Second component	Colour of alcoholic solution	Composition of compound formed	Colour and crystalline form of compound	m.p. °C. (uncorr.)	N analysis by Dumas*
Dinitro-*o*-cresol	Aniline	Deep red	1 : 1	Bright orange needles†	65– 67	14·45 (14·44)
	Pyridine	Deep yellow	1 : 1	Bright yellow needles†	95– 96‡	15·26 (15·17)
	p-Toluidine	Deep orange red	1 : 1	Orange needles	94– 95	13·75 (13·77)
	N N-dimethyl-*p*-toluidine	Deep red	1 : 1	Bright orange needles	71– 72	12·81 (12·61)
	Indole	Orange	1 : 1	Orange yellow needles	119–120	13·50 (13·34)
	Nicotine	Orange	2 : 1 of base	Deep yellow prisms	120–122	15·18 (15·06)§
	α-Naphthylamine	Deep red	1 : 1	Reddish brown needles	135·5–137	12·52 (12·31)
	β-Naphthylamine	Deep red	1 : 1	Deep red needles	86	12·38 (12·31)
	Quinoline	Orange yellow	1 : 1	Orange needles	114	12·94 (12·85)
	Acridine	Deep yellow	1 : 1	Orange yellow needles	168	11·26 (11·14)
	Di-β-naphthylamine	Orange red	2 : 1 of base	Brick red plates	98	10·53 (10·53)‖
	Nitron	Yellow	1 : 1	Bright yellow needles	219¶	16·48 (16·47)
	Strychnine	Deep yellow	1 : 1	Bright yellow rosettes	231– 33	10·64 (10·53)
	Brucine	Deep yellow	1 : 1	Shining yellow plates	254–255 (*d*)	9·26 (9·46)
	Naphthalene	Bright yellow	1 : 1	Lemon yellow needles	92– 93	8·73 (8·59)
	Acenaphthene	Yellow	1 : 1	Yellow needles	108–109	8·08 (7·96)
	Phenanthrene	Yellow	1 : 1	Pale yellow needles	78– 80	7·53 (7·45)
	α-Naphthol	Orange	1 : 1	Orange yellow needles	142–144	8·19 (8·19)
Dinitro-*o*-*cyclo*hexyl-phenol	α-Naphthylamine	Deep red	1 : 1	Dark brown needles	87	10·32 (10·27)
	Nicotine	Orange brown	1 : 1	Bright orange yellow needles	153–154	13·26 (13·09)
	Nitron	Deep red	1 : 1	Orange red needles	206–207	14·71 (14·54)
	Naphthalene	Yellow	1 : 1	Lemon yellow needles	71– 72	7·26 (7·11)

* The values in parentheses are the theoretical percentages. † Decomposed slowly on standing in air with loss of the basic constituent. ‡ Gibson, *J. chem. Soc.* 1925, p. 45, gives m.p. 96°C. § The 1 : 1 compound requires *N* 15·56 %. ‖ The 1 : 1 compound requires *N* 9·00 %. ¶ Recrystallized from alcohol.

The liberation of iodine is a time reaction, the rate of which was found to be influenced by the excess of iodide-iodate employed. Under certain conditions it is possible to estimate D.N.C. within an accuracy of 2% by immediate titration of the liberated iodine with thiosulphate (Table 2). Alternatively, if an excess of thiosulphate is added after the iodide-iodate, the excess then being determined by back titration with standard iodine, the error in the estimation is less than 1% (Table 2). No blank experiment is necessary in either case.

TABLE 2. *Estimation of dinitro-o-cresol*

Sample	% D.N.C. found: Direct thiosulphate titration	Back titration with iodine	Gravimetric estimation with nitron	m.p. of nitron complex °C. (uncorr.)
Pure D.N.C.	98·6	99·6	98·8	218–19
(m.p. 85·5°C.)	98·7	99·7	98·8	218–19
	99·1	99·9		
K1 (moist)	86·7	88·4	86·6	218–19
K2 (moist)	87·3	89·0	87·4	217·5–18
E.B.	96·7	98·9	96·5	216–18

The presence of phenols does not interfere with the estimation (Table 3), but mineral acid, if present, must be removed as described below.

TABLE 3. *Effect of phenol and* m-*cresol on the volumetric estimation of* D.N.C. (*back titration method*)

Material	% D.N.C. found
Pure D.N.C. (m.p. 85·5°C.)	99·7
" + equal wt. of phenol	99·5
" + equal wt. of *m*-cresol	99·6
" + equal wts. of phenol and of *m*-cresol	99·1

The method is equally applicable to the estimation of dinitro-*o*-*cyclo*hexylphenol (Table 4).

TABLE 4. *Estimation of pure dinitro*-o-cyclo*hexylphenol*

Method	% D.N.O.C.H.P. found
Direct thiosulphate titration	96·3
	96·7
Back titration	99·9
	99·7
	100·1
Gravimetrically as nitron complex	98·3 (m.p. 206–207°C.)
	98·4 (m.p. 205–206·5°C.)

Solutions required

N/10 *sodium thiosulphate*. Standardized against *N*/10 potassium iodate.

N/10 *iodine*. Standardized against the thiosulphate solution.

N/2 *iodide-iodate*. Containing 17·835 g. potassium iodate and 70 g. potassium iodide/l. The solution is usually slightly brown, and immediately before use should be treated with *N*/10 thiosulphate, drop by drop, until colourless.

D.N.C. *solution.* About 2·0 g. of the sample, free from mineral acid, is weighed out accurately and made up to 250 ml. with absolute alcohol. If mineral acid is present, a weighed quantity (about 2 g.) is dissolved in about 500 ml. of ether in a separating funnel and the solution is shaken with 10 ml. portions of distilled water until the aqueous washings are neutral to litmus. The ether solution is then dried over anhydrous sodium sulphate, filtered and the ether removed. The resulting cresol, freed from acid, is dissolved in absolute alcohol and made up to 250 ml. In removing mineral acid by this method the loss of D.N.C. is negligible.

Procedure

(*a*) *Direct titration.* 25 ml. of the alcoholic solution is pipetted into a conical flask and 50 ml. iodide-iodate added. Sufficient distilled water (about 25 ml.) must now be added to dissolve the yellow potassium salt of D.N.C. which crystallizes out. The clear reddish brown solution is then titrated immediately with thiosulphate using starch indicator. The end-point is sharp and occurs when the greyish blue solution changes to clear orange. 1 ml. $N/10$ thiosulphate = 0·01981 g. D.N.C.

(*b*) *Back titration.* 25 ml. of the D.N.C. solution is treated with 10 ml. iodide-iodate and distilled water is added until the solution is clear. 20 ml. $N/10$ thiosulphate is now added. The excess thiosulphate present is then determined by back titration with $N/10$ iodine, starch being added just before the end-point. If this titre is x ml., then $(20 - x)$ = ml. $N/10$ thiosulphate reacting with the liberated iodine and 1 ml. $N/10$ thiosulphate = 0·01981 g. D.N.C.

(2) *Gravimetric method*

For this purpose use has been made of the tendency of D.N.C. to form molecular compounds. In most cases, these only crystallize out from the alcohol solution when the two components are present in carefully adjusted quantities. Such complexes are unsuitable for use in a gravimetric estimation. Various bases of high molecular weight, however, combine readily to form sparingly soluble compounds with D.N.C., and of those examined (Tables 2 and 5) nitron and acridine can be used in a gravimetric estimation when the compound formation is performed under special conditions. The error in these estimations is slightly greater than that of the volumetric method but has the advantage that the purity of the weighed complex can be checked at once by melting-point determination. The presence of complexes of other polynitro compounds with the base would result in a depression of the melting point, and the method, given the correct sharp melting-point, is therefore specific to D.N.C.

Compound formation between dinitro-*o*-*cyclo*hexylphenol and nitron has also been studied and applied to give a method for the gravimetric estimation of this substance (Table 4).

Procedure

20 ml. of the solution of D.N.C.* in alcohol made up as described above is pipetted into a tall 400 ml. beaker, heated to boiling on the water-bath and hot distilled water slowly added until incipient precipitation (about 180 ml.). When boiling, 5 ml. of a filtered solu-

* Bromides, iodides, nitrites, nitrates, chromates, chlorates, perchlorates, thiocyanates and oxalates yield insoluble compounds with nitron and must not be present.

tion of 10% nitron in 4% acetic acid is run in and the mixture well stirred with a glass rod fitted with a rubber 'policeman'. The molecular compound begins to separate almost immediately in the form of small yellow needles. After standing overnight the complex is filtered into a tared Gooch crucible and washed with about 50 ml. distilled water. After drying in the air oven at 100° for at least 6 hr., the crucible is reweighed and the melting-point of the complex is taken. Then wt. D.N.C. = wt. complex × 0·3883.

Table 5 gives the results obtained with four other bases used in the gravimetric estimation of D.N.C. In each case the D.N.C. solution and procedure were as given above.

TABLE 5. *Gravimetric estimation of pure* D.N.C.

Other component	Reagent employed	% D.N.C.	m.p. of complex °C. (uncorr.)
Brucine	10 ml. 5% base in 5% HAc	66·3	184–223 (*d*)
α-Naphthylamine	10 ml. 5% ,, 40% HAc	93·8	135–137
Indole	10 ml. 5% ,, 50% HAc	93·9	119–120
Acridine	10 ml. 5% ,, 10% HAc	98·5	168

*Estimation of dinitro-*o*-cyclo**hexylphenol*

The solution for analysis was made up by dissolving 1·400 g. of the pure phenol (m.p. 105·5° C.) in 250 ml. absolute alcohol.

Volumetric estimation. 50 ml. portions of the alcoholic solution were used following the procedure described above. The end-point was not quite so distinct as in the D.N.C. estimation. 1 ml. *N*/10 thiosulphate = 0·02661 g. D.N.O.C.H.P.

Gravimetric estimation. 50 ml. of the boiling alcoholic solution to which 80 ml. hot distilled water had been added was treated while boiling with 5 ml. 10% nitron in 4% acetic acid. Wt. D.N.O.C.H.P. = wt. complex × 0·4603.

SUMMARY

Previous work on the estimation of dinitro-*o*-cresol and related substances and their use as insecticides is reviewed. A number of new derivatives of dinitro-*o*-cresol and dinitro-*o*-*cyclo*hexylphenol and two routine methods of estimating these substances are described.

The author gratefully acknowledges the advice and helpful criticism given by Dr H. Martin during the course of this investigation. He is also indebted to Mr R. F. Batt who has performed much of the analytical work.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

The Annual General Meeting of the Association was held at 11 a.m. on Friday, 20 Feb. 1942, in the London School of Hygiene and Tropical Medicine, the President, Dr H. Martin, in the Chair. After the formal business there was held a *Symposium on domestic entomology* in which the following papers were read:

I. New methods of controlling head and body lice (with film). By J. R. Busvine.
II. Experimental work on the transmission and development of scabies. By K. Mellanby.
III. Some quantitative aspects of scabies parasitology. By C. G. Johnson.
IV. The control of flies in town and country. By H. G. H. Kearns.
V. Furniture beetles and other wood-borers. By R. C. Fisher.
VI. Invasion of houses by ants and earwigs. By G. Fox Wilson.

I. NEW METHODS OF CONTROLLING HEAD AND BODY LICE

By J. R. Busvine, *The Bureau of Entomology, 41 Queen's Gate, London, S.W. 7*

Abstract. Some recent applied research was described which seems to provide a safeguard against epidemics of lice and the terrible diseases associated with them. While people are all able to get regular changes of under-garments, the body louse is not likely to spread rapidly, and occasional cases of infestation can be dealt with by improvements on methods used during the last war. At any time, however, unhygienic conditions may arise as a result of severe bombing, invasion or other hazards of 'total' war which may favour the louse. If this pest becomes widespread it is exceedingly hard to eradicate, as was found in Flanders during the last war and in other subsequent epidemics. The difficulty is that deloused persons rapidly become reinfested; but this can now be overcome by the use of lasting insecticides, applied in various ingenious ways, which 'proof' a person for a month after treatment. These methods, which have been thoroughly tested on verminous men, were illustrated by a short amateur film.

It was also pointed out that the head louse, which is much more common, is closely allied to the body louse and may possibly be a disease vector. Similar lasting insecticides are of value for this pest, applied as 'medicated' hair dressings.

II. EXPERIMENTAL WORK ON THE TRANSMISSION AND DEVELOPMENT OF SCABIES

By K. Mellanby, *The Sorby Research Institute, 18 Oakholme Road, Sheffield 10*

Abstract. Experiments were described concerning transmission of scabies. Twelve volunteers had lived under controlled conditions for over a year and submitted themselves to contacts of various kinds. It appears that the disease is seldom transmitted by the agency of inanimate objects (blankets, clothing, etc.), but is readily transmitted by personal contact, particularly between individuals sharing a bed. 'Control' individuals have lived together in the same house with the infested volunteers, and the ordinary contacts of social life would appear to have been insufficient to cause scabies to be transmitted on any occasion.

Some particulars of the life history and biology of *Sarcoptes* were given and illustrated by lantern slides made from microphotographs of whole mounts and serial sections of biopsies taken from scabies patients.

III. SOME QUANTITATIVE ASPECTS OF SCABIES PARASITOLOGY

By C. G. Johnson, *The Sorby Research Institute, 18 Oakholme Road, Sheffield 10*

Abstract. An account was given of research work on scabies parasitology from the quantitative aspect. Contrary to popular belief most scabies cases suffer from an exceedingly low parasitic infection. A technique by which all ovigerous females of *Sarcoptes* are removed from the patients shows that the average of ovigerous females per patient (soldiers) is only 14: 52% of the cases have six or less ovigerous females, 76% of the cases have 14 or less, and only 12% of the cases have more than 30 ovigerous females. Occasionally a patient with more than 100 is found but this is exceedingly rare. The reproductive potential of the *Sarcoptes* is enormous, but natural infestations rarely show a high parasite count, owing to high mortality among the itch mites. Infestations may die out spontaneously. An analysis has been made of the sites of the body most frequently infested. Of these, the hands and the wrists is the commonest site and 85% of all cases are infected there: over 50% of the total mites removed have come from this site. This work is in its early stages and insufficient data make it impossible to give, at present, an integrated picture of the epidemiology of the disease.

IV. THE CONTROL OF FLIES IN COUNTRY AND TOWN

By H. G. H. Kearns, *Research Station, Long Ashton, Bristol*

A number of species of dipterous flies are the cause of considerable annoyance and some loss of food and health in rural districts as well as in the suburbs of country towns. War-time conditions and the bombing of cities introduce special problems when stores of foodstuffs are damaged and left exposed to the elements for any length of time.

Stinging flies. Everyone familiar with country life has experienced stings from the minute midges, *Ceratopogon bipunctatus* or *C. varius*, and from the horse-fly or cleg, *Haematopota pluvialis*.

Ceratopogon breeds in decaying moist vegetation and frequents hedgerows, trees, rotting humus piles in gardens, etc. It occurs more or less throughout the summer, but is particularly prevalent in a spell of warm weather following a period of rain. It will attack at any time of the day, but is most active during the late afternoon and evening. The flies appear partial to skin covered with hair, and often the back of the neck and head is severely attacked. The puncture is made so gently that it is unnoticed. It is the injected fluid that appears to cause the irritation which is evident almost immediately after the midge has finished feeding. Most people are equally attractive to the midge, but there are considerable differences in the reaction of individuals to the bites. Thus a city worker who has not been hardened by exposure to the sun suffers considerably if suddenly exposed to attack. On the other hand, the seasoned farm hand whose skin is sun baked and toughened by continual outdoor life seems to suffer little or no irritation. It is not clear whether resistance is due simply to skin texture or to other factors. Dozens of stings are often acquired in a short space of time, the sum total resulting in considerable discomfort. The area around the puncture is slightly inflamed and swollen, but the irritation does not as a rule persist for more than a short while unless aggravated by rubbing and scratching.

The midges are fairly readily repelled by lightly rubbing over the exposed parts with pyrethrum extract dissolved in highly refined petroleum oil of the type and viscosity used for the preparation of many brilliantines, or alternatively, in olive oil which is used as the basis of many sun-bathing mixtures. The concentration of pyrethrins should be about 1·0%, i.e. the extract from 100 g. of good-quality flowers made up to 100 c.c. with oil. The petroleum-oil dressing is perhaps more suitable for the hair and the olive oil for the skin. It is only necessary to rub the mixture lightly over the skin and the duration of effective repellent action lasts about 5 hr., but this is considerably reduced by profuse perspiration.

A more lasting and nearly equally effective preparation can be prepared by substituting for the pyrethrum extract dodecyl thiocyanate applied at 10% concentration or other organic thiocyanate such as *n*-butyl carbitol thiocyanate (known as 'Lethane 384') at 20%. The earlier 'Lethanes' had such an objectionable odour that they were repellent to human beings as well as to the midges. It is probable that the more recently introduced material possessing little odour known as 'Lethane 384 Special' applied at 20% concentration would be effective. The concentration in the petroleum or olive oils should be 10% of active constituents (thiocyanates). There appears to be in this country

only a very slight risk of dermatitis or skin irritation from these preparations. The action of the dressings is to irritate the feet of the flies so that they fail to obtain sufficient foothold to insert the mouth-parts into the skin. Aromatic essential oils, such as citronella, lavender, etc., are of only slight value and considerably inferior to the repellents described.

The horse-flies or clegs (*Haematopota* sp.) are exceptionally vicious; they occur in most parts of this country, but generally it is only on low-lying land intersected with dykes, rhines and other drainage canals that they occur in such numbers that they seriously inconvenience farm labourers and others. On warm, sunny days the flies suddenly alight on the backs of the hands, on the wrists, arms, neck and even puncture the skin through socks and other loosely woven clothing. The insertion of the glandular product from the stylets into the skin feels like the entry of a blunt needle. Most individuals seem equally susceptible to attack, but the reaction to the sting varies considerably; in many cases only a slight swelling results which irritates for 2 or 3 days, whereas in others the irritation may last for 10 or more days; in extreme cases very extensive swelling occurs, for example, on the back of the hands at the point of insertion of the fingers. It appears that within a season individuals can acquire some resistance to the effects of the stings. In some seasons in parts of Somerset, such as the low-lying basket-willow beds, the flies are so numerous and their attacks so persistent in hot weather that some farm hands refuse to continue to work during the full heat of the day.

The repellents successfully used for the midges fail completely with this fly. The sustained application of cotton-wool saturated in witch hazel gives the best relief from a sting. The use of the numerous country remedies such as blue-bag, ammonia, crushed dock leaves, etc., are mainly of psychological value only.

Cluster flies. In many parts of the country, more particularly in the west where pastureland predominates, a number of species of flies has the characteristic feature of swarming in autumn in hollow trees and buildings. The principal species are muscids, e.g. *Pollenia rudis*, *Musca autumnalis*, *Pyrellia lasiopthalma* and the chloropetid, *Chloropisca circumdata*. Other species that commonly occur in small numbers associated with swarms of the muscids already mentioned are *Musca domestica*, *M. stabulans* and *Stomoxys calcitrans*. At times numbers of chalcids accompany the flies.

The swarming of the muscids is an act of hibernation in autumn, whereas, with the chloropetid, it may also occur in the summer. A characteristic feature of the muscid swarms is that the same site is often selected for hibernation year after year, and so persistent is the habit in the west country that certain country houses are recognized as fly-infested houses. This habit often persists despite the fact that the districts become built up and therefore offer the flies a wider choice of winter quarters, yet the new houses remain comparatively free from infestation. The data collected on infested buildings over a period of some years are most conflicting. In some buildings during the winter the flies have been nearly eliminated by control measures, and in several instances the eliminated species has been replaced by another in the following year. With *Pollenia rudis* it appears that houses and buildings most subject to infestation are relatively isolated and occur on the crest of rising pasture ground having a high earthworm population. The larvae of this species are parasitic on earthworms.

The first signs of an infestation occur in late July or early August when a few flies are noticed basking in the sun on the outside of the windows. The numbers generally gradually increase, and as soon as cold evenings occur they make every effort to enter the building. Eventually, when the first frost comes, huge numbers of flies may enter the house through spaces between tiles, eaves, loose-fitting windows, etc. So persistent are their efforts to find shelter that they will descend down unused chimney stacks, and it is impracticable to prevent their entry. Sunlight and warmth stimulate them, and it is usual for them to bask in the sun until as late as early November. Their natural habitat for hibernation is a dry, sheltered, hollow tree which keeps their metabolism at a low level and avoids undue waste of their extensive fat body. Therefore, cool and dry roof spaces of churches, clock towers, private houses and other positions offering similar environment are well suited to their requirements. They frequently cluster together one row thick in patches up to $\frac{3}{4}$ sq. ft. in size. Their entry into living rooms of a house is accidental and frequently fatal to them because it may keep them for weeks in a semi-torpid condition and while they are in this condition they are a source of annoyance to the inmates of the house. Ultimately they die from exhaustion.

As soon as the warmer weather arrives in March, numbers of the flies are stimulated to leave their hibernation quarters and they bask in the sun on the tiles or slates of the building and at this stage numbers are consumed by birds, and at times the presence of birds can be used as an indicator of infested houses. From this time onwards the flies become a nuisance to the inmates of houses because they still use the house for protection until about April and frequently get into living rooms.

The flies are readily killed by atomizing pyrethrum extract or one of the organic thiocyanates previously mentioned dissolved in kerosene. The kerosene should be of low aromatic content and odourless. The grade frequently used in fly sprays is often objectionable in odour, and if liberally used may cause a form of ophthalmia, nasal catarrh and feeling of sickness. The kerosene may be replaced by highly refined petroleum oil provided the oily deposit is of no consequence. The concentration of the toxic material varies according to the degree of atomization, the finer and more even the particle size the higher the concentration of the toxic material. For use in hand atomizers and low-pressure paint spray guns 0·25–0·5% total pyrethrins or 4% of thiocyanates (e.g. 8% 'Lethane 384 Special') is satisfactory.

The flies in small and medium-sized living rooms are readily killed by atomizing the insecticide by means of a small compressor outfit or even by the use of a properly designed hand 'Flit' type gun. The average Flit type of hand atomizer is usually unsatisfactory, as it fails to atomize sufficiently and it deposits liquid on furniture and fabrics. Unfortunately, a single application is useless because the incoming flies in autumn arrive in a succession of batches over a period of 2–8 weeks. If the flies inadvertently congregate in living rooms, it may be necessary to spray daily to keep them down to reasonable numbers. Under such circumstances a small electric-driven self-contained atomizer has been found invaluable, as it can be left unattended for a predetermined length of time to give the necessary dosage of insecticide.

If the majority of the flies can be killed during hibernation the annoyance of the emergence in spring is largely avoided, and in about 70% of the infestations under observation the next winter's infestation has been markedly reduced. The elimination of the flies in their hibernating quarters often presents considerable technical difficulties. It is essential to submit the flies to a lethal concentration of the insecticide, and the greatest difficulty is met in attaining the necessary concentration of insecticide in large buildings and roof spaces due to dilution by air draughts. Satisfactory results have been obtained in churches with modified air and liquid jets of paint spray guns so that a very large ratio of compressed air to liquid is arranged. Hundreds of thousands of flies have been killed in the open roof spaces of a church with 2 pt. of pyrethrum kerosene (0·5 total pyrethrins) per 100,000 cu. ft. of air space atomized from floor level. In some buildings the flies may hibernate in inaccessible positions, such as between the boarding and bitumen felt of the roof, and such cases demand special treatment. The spring emergence is dealt with by the same means as suggested for the autumn.

Indirect methods of control are very limited, but in some instances the provision of easy entry and exit, such as skylights in roofs and walls of west and south-west aspects, has proven well worth while. The reduction of earthworms, which are the host of the larvae of *Pollenia*, in nearby lawns by means of mowrah meal dressings is another example. The application of repellent materials to roof timbers has proven unsatisfactory.

Flies in towns. In towns the replacement of the horse by the internal combustion engine, and the consequent absence of horse manure, together with the greatly improved methods of collecting and disposing of household garbage has practically eliminated the house-fly as a serious nuisance. There are still industries dealing with animal organic matter, situated in towns, that give rise to local infestation of blow-flies. This nuisance could be controlled by better organized methods of handling the decomposing materials.

War has introduced special fly problems in some of the bombed towns and cities. Food stores of all kinds have been damaged by high explosives and fire, and for unavoidable reasons quantities of food varying widely in composition have been left exposed to the elements for months. Tinned foodstuffs developed rust pin-holes and rats enlarged the holes and scattered the contents. The food was frequently mixed with bricks, mortar, sheet iron, steel girders, etc. In this medley several species of flies developed in vast numbers. Thus, in one city, the infestation of *Calliphora* and *Lucilia* blow-flies was so great that the fish market had to be closed early in the day and it was impossible to expose meat or fish in the shops. Business premises and shops in the vicinity of breeding sites were infested by hundreds of thousands of flies. In another instance an entire suburb was infested with house-flies.

The obvious method of control was to clear up the breeding sites by removing all the debris. However, this procedure takes much labour, transport and time. It was, therefore, necessary to keep the flies within bounds in the breeding sites and eliminate them as far as possible from buildings. Liberal applications of a tar-oil emulsion to the surfaces of all the piles of debris containing foodstuffs proved to be the most economical and satisfactory method of retarding the breeding of the flies. A wash was prepared by emulsifying a neutral high-boiling grade A tar oil by means of sulphite lye,

and applying it at 2·0% concentration of oil. A neutral oil and emulsifier were selected, as it reduced the liability of the operators in hot weather to skin soreness. The wash was applied throughout the summer by means of spray guns fed from mobile high-pressure hydraulic pumps. The amount of wash applied varied according to the nature of the site. The tar oil acted mainly as a repellent to egg-laying females, although in addition it killed up to 90% of the eggs with which it came into contact. It was found necessary to spray each of the sites every 7–10 days as the tar oil under summer conditions volatilized rapidly, and two applications were required before any marked drop in the breeding capacity of the site was noted. Concurrent with the spraying the sites were cleared and every day the exposed area was sprayed to prevent or retard egg laying by flies.

The infestations in buildings and houses were efficiently dealt with by means of a portable compressor mounted on a car-trailer chassis. The air line was led into the building and a suitable quantity of insecticide atomized in the rooms. 'Lethane 384 Special' at 7·5% concentration dissolved in odourless kerosene or pyrethrum extract in kerosene at 0·25% total pyrethrins provided satisfactory results.

V. FURNITURE BEETLES AND OTHER WOOD-BORERS

By Ronald C. Fisher, *Forest Products Research Laboratory, Princes Risborough, Bucks*

In a symposium of this kind it is obviously impossible to give a complete account of the biology and methods of control of the insects which attack structural timbers and furniture in houses and other buildings. Whilst I wish to avoid a mere catalogue of names and condensed accounts of life histories, so far as they are known, it is important to mention which insects are primarily concerned and give some account of the conditions required for their development and increase, as well as comment upon their control.

Termites or white ants, so injurious in tropical and subtropical countries, are of no account in this country, and need not be considered in the present discussion. The commonest household wood-borers are the so-called furniture beetles of the family Anobiidae, and include the death-watch beetle, *Xestobium rufovillosum*, and the common furniture beetle, *Anobium punctatum*. Two other species—*Ernobius mollis*, sometimes found on the edges of coniferous timbers from which the bark has not been removed, and *Ptilinus pectinicornis*, occasionally found in beech furniture—are of less importance and not so frequent in their occurrence.

The death-watch beetle. This insect is not found in many houses, although frequent requests are received at Princes Risborough from householders for advice on its control, because it is said to have been heard in panelling, under a floor or in some other part of a house. It is often held responsible for any type of insect damage to woodwork in houses. The tapping for which it is notorious is confused most probably with the ticking noise which can be caused by book lice (psocids). The death-watch beetle larva is, however, often responsible for serious damage to structural timbers or sometimes panelling in old houses, as well as in churches, in which old oak timbers are present. I mention this insect now because it provides an interesting example of the importance of an accurate knowledge of the food requirements of a wood-borer and of the environmental conditions necessary for its development, in relation to methods of prevention and control. This beetle will not infest sound oak, but fungal decay renders timber liable to attack, and a high moisture content of decayed wood accelerates the rate of development of the larva. The mechanism of the effect of decay in timber upon the metabolism of the larva of the death-watch beetle has been studied by my colleague, Mr Campbell, of the Wood Chemistry Section, who has recently reinvestigated this problem in the light of our biological observations and experiments. He has come to the conclusion that decayed timber is more suitable for the insect than sound wood, because it is easier to bore through, and because of a slight but significant increase in the nitrogen content of the wood as a result of its decayed condition. As decay increases, the nitrogenous material becomes more readily available to the larva by increased boring, and it therefore grows faster, and completes its development in a shorter period in more decayed timber.

So far as control measures are concerned, damage by the death-watch beetle can be avoided by preventing conditions which would give rise to fungal attack. At the present time there is a unique opportunity of ensuring that in the reconstruction of bombed churches or in the erection of new houses and buildings, this insect need, in the future, no longer be a cause of concern if simple structural precautions are taken to prevent fungal attack, and care is taken not to introduce the

insect in untreated timbers from other old buildings. The treatment of timber already infested is quite another matter, and a much more difficult problem.

The common furniture beetle. This insect, by far the commonest cause of 'wormy' woodwork in houses, is probably present in most, in some old article of furniture, in a discarded wicker chair, in the sapwood of the roofing timbers, floor-boards, joists or panelling. On an average, 25 % of the inquiries dealt with each year at Princes Risborough on insect attack concern this beetle. Like many common pests, accurate information upon its biology and, in particular, upon its food requirements, is very meagre and incomplete, and up to the present, control measures have been based largely on age-old recommendations, which do not offer any real hope of complete success. A new study of this insect from the point of view of its food relationships is required, and has now been started. Similar investigations have recently been undertaken in Germany, where *Anobium punctatum* is also a common pest. The results so far obtained are interesting, and there are indications that this so-called 'dry-wood borer' likes moisture in wood. Where the moisture content of wood is low, as for instance in furniture stored indoors in heated rooms, the present indication is that the progress of *Anobium* attack is extremely slow and the life cycle prolonged over several years. In wood where the moisture content is higher, as in structural timbers in roofs, in damp cellars, in farm sheds and out-buildings, where the insect is often a serious pest, the life cycle is probably much shorter.

Anobium is of much commoner occurrence than the death-watch beetle, and attacks the sapwood chiefly, of most timbers, both coniferous and hardwood species. A notable exception are the eucalypts of Australia, which are immune from infestation, whilst certain other woods in that country are liable to attack. Why this should be and what constitutes the food of the larva of *Anobium*, with a possible preference for some timbers, or timbers in a specific condition as regards chemical composition, botanical structure, age, i.e. time after felling, or the presence or absence of fungal decay, are at present more or less unknown.

An important problem arises out of the observed suitability of plywood for *Anobium* attack, especially in these days when it is being used to an ever-increasing extent in place of solid wood. Frequent instances have come to our notice of severe damage to plywood panelling in partitions, doors, picture frames, etc., and the effective treatment of this type of infested wood is particularly difficult. At present, preventive treatments are not considered worth while by manufacturers, or builders, no doubt because *Anobium* does not make its appearance in their products until long after these have left their premises, and even many years after they have been put into position in houses. Under present war-time conditions, when household furniture from bombed houses or vacated premises is often stored in barns and other buildings in which *Anobium* is present, there is a risk of spread of infestation to the furniture, but evidence of such attack may not become apparent at once. Moreover, in common with other insects, *Anobium* may have an opportunity of multiplying and spreading in unoccupied houses where heating is absent and damp conditions prevail. These are problems and risks which have to be taken in war-time, but are usually entirely overlooked by the majority of householders and those immediately concerned in storage of furniture. As the true extent of the damage will not become apparent for several years, the general attitude has been 'why worry'! For those who do concern themselves with the preservation of their property the prevention of 'worm' damage in furniture is worth attention now.

Lyctus *powder-post beetles.* Passing mention must be made of *Lyctus* powder-post beetles, which cause injury to the sapwood only of hardwoods and occur in new furniture or in the sapwood of recently erected oak beams, in panelling of walnut or oak. The presence of these insects is usually due to the use of wormy wood by the manufacturers. Treatment of infested wood after use is again difficult, but damage by these insects can be entirely prevented by the omission of sapwood, which contains starch, the principal food of the larvae. Within recent years extensive research has been carried on to determine methods of producing starch-free and, therefore, *Lyctus*-immune timber for the use of manufacturers and builders. The results of this work by Wilson (1933), Parkin & Phillips (1938, 1939) have been published in the *Annals of Applied Biology* and in *Forestry*.

The house longhorn beetle. A word is necessary on the status of the house longhorn beetle, *Hylotrupes bajulus*, as a pest in this country. In the Baltic countries, Sweden, Denmark, north and central Germany, this insect is the most important pest of softwood structural timbers, and during the past 20 years has been responsible for extensive damage to houses in these countries. In some districts treatment of infested buildings is compulsory, and a system of insurance against infestation is in operation.

Research, particularly in Germany, has shown that the recent marked increase in damage by *Hylotrupes* is due to the use of immature timber, of less than 20 years old, consisting almost entirely

of sapwood, which is preferred by the insect to heartwood. It is particularly interesting to note that, in 1941, Wolff of the Forest Research Station, Eberswalde, near Berlin, where much of the research has been carried out, wrote that 'the need for the preservation of timber against infestation is urgent, particularly because of the cheap methods of construction and the use of inferior and unseasoned wood. Adequate measures for new buildings must be planned quickly.'

In this country *Hylotrupes* occurs only rarely at present. In 1934 Dr Parkin summarized the three cases which had then come to our notice at Princes Risborough. Since that date a further nine have been recorded, some in old buildings where it was impossible to trace the origin of attack and others in imported packing cases, which may have been the most probable origin of the insect reaching this country. Under present conditions, when widespread felling of our woodlands is proceeding and unseasoned timber is being used, the possibility of trouble with *Hylotrupes* some years hence cannot be overlooked, and it is for this reason that the progress of work on the biology, physiology and control of this important pest in Germany is being followed with interest.

Other wood-borers. In addition to the insects mentioned above, there are several others which occur from time to time in woodwork in buildings. The *Sirex* woodwasps, for instance, are primarily forest insects, but sometimes emerge from built-in timbers in new houses, causing more alarm than damage. They have been the subject of recent work by my colleagues, Mr Cartwright of the Mycology Section and Dr Parkin, upon the relationship which exists between these insects and fungus in infested wood. Their findings constitute another instance of the close association between fungus and wood-boring insects, which other investigations on timber insects have shown to be of more general occurrence than was hitherto believed.

The control of wood-boring insects

Remedial measures. Mention has been made of the value of preventive treatments for the control of the above insects, but once attack has taken place, by far the most common occurrence in houses, treatment becomes difficult, and all the possible methods are bound up with the complications involved by the insects living in wood. This is difficult to penetrate with insecticides and often difficult even to reach when structural timbers are involved. The following is a very brief summary of different methods of treatment. The most suitable for any individual case of infestation can be decided upon only after careful consideration of the conditions present: there is no universal remedy:

(1) *Insecticide treatments*—applied by brush or spray, giving some degree of control, but not entirely satisfactory. The amount of discoloration which may result, possible injury to the finish of the furniture, or an unpleasant and lasting odour, are amongst the factors which have to be taken into consideration and which under certain circumstances may prevent the use of an insecticide.

(2) *Fumigation*—at present largely limited to articles which can be easily handled and treated in a fumigating chamber. HCN is usually used, and the treatment can be applied only by experts; the value of other fumigants has yet to be examined. There is a wide scope for investigation of the use of fumigation for the control of the common furniture beetle, particularly in plywood, where the dimensions of the timber are small and where penetration of insecticides is even more difficult or uncertain than in solid wood. An added complication in fumigation is the power of the timber to retain the fumigant and the danger arising subsequently from the gas gradually coming off and building up a lethal concentration in a room where ventilation is poor.

(3) *Heat treatments*—heat sterilization of articles of furniture is a possibility where facilities are easily available. A modification of this method has been used with success for the destruction of the house longhorn beetle in structural timbers in buildings on the Continent. Some experimental work on its use for the treatment of the timbers of an old oak barn, attacked by the common furniture beetle, has been carried out in this country.

Whilst attention has been devoted to the methods of control outlined above, extensive experimental work has been carried out by my colleague, Mr Cann, on the use of preservatives for the prevention of attack by *Lyctus* powder-post beetles in timber. A standard type of insecticide test, which is required for the comparison of the toxicity of different chemicals or preparations, as is undertaken in the case of fungicides, has, however, not been developed so far, largely because our knowledge of the optimum conditions in wood for the insects is as yet incomplete. Once such information becomes available, in particular for the common furniture beetle, this type of work can be developed and extended to examine the degree of susceptibility to attack by wood-boring and other insects, of different types of wallboards and such materials containing cellulose, which are being used more and more in place of solid timber or plywood.

Non-wood-borers in houses. In conclusion, I would direct your attention for a moment or two to a number of other insects which are not wood-borers, but which are associated with wood, and may occur in large numbers, causing much concern by their mere presence. The lathridiid and *Cryptophagus* beetles, the so-called plaster beetles, and the small swiftly moving psocids or book lice, all of which live on moulds or other fungi, growing on the surface of damp plaster, unseasoned wood or plywood, frequently occur in houses and cause considerable alarm. Recent papers by Hinton of the Natural History Museum provide much-needed data on the plaster beetles. All these insects, which occur most frequently in new buildings, only partly dried, will disappear as they dry out; remedial measures other than providing facilities for airing and quick drying of new houses are not usually necessary.

The larvae of some species of clothes moth frequently breed in dust and debris, in cracks and crevices between floorboards, behind skirting-boards, and are difficult to eradicate. In fact these insects constitute another serious war-time problem in unoccupied houses, and I often wonder whose business it is to deal with this, and if the damage caused is fully realized: in the aggregate, it must be considerable.

Finally, serious trouble can result from infestation by the furniture mite *Glycyphagus domesticus*, a species which occurs in small numbers in many houses, but which in damp premises may appear as a mass invasion often originating in furniture stuffing of certain types. Few cases have been heard of lately, but a serious outbreak occurred in 1926–8 in Bristol and district. An account of the biology of this pest is given by Hora (1934) in the *Annals of Applied Biology*, who has discussed methods of control.

Summary

On the physiological side, important additions have been made during the past 10–15 years to the knowledge of the feeding habits of wood-boring insects, which, according to Uvarov (1928) in his summary of the literature on insect nutrition, 'must be classified as insects of absolutely unknown feeding habits'. Much has yet to be unravelled, even with our common species, but the work so far accomplished has already pointed the way to effective methods of dealing with some of these domestic pests.

For the prevention of infestation, the value of simple structural precautions in buildings has been demonstrated, and the need for an increased use of preservatives or deterrents became apparent. So far, there has been little advance in this direction, for seldom, if ever, are building timbers for indoor use treated in this country to withstand attack by insects or fungi. Treatments of any kind are not even considered until infestation has taken place and is often well advanced, by which time effective remedial measures are all the more difficult to apply, and may even not be worth while, replacement with sound wood being all that is practically possible.

So far as the treatment of timber already infested is concerned, whether in the structure of a building or in furniture, the old methods of spraying and painting on insecticides are still relied upon to a great extent, and provided they are applied with perseverance and repeated at intervals, have certainly a definite value. Fumigation offers other possibilities, but requires further investigation, and heat sterilization is applicable in particular circumstances. Whatever the method used, the difficulties inherent in having to contend with the properties of wood as well as the powers of resistance of the insects, form the greatest obstacle to the development of simple methods of effective treatment.

VI. THE INVASION OF HOUSES BY EARWIGS AND ANTS

By G. Fox Wilson, *Royal Horticultural Society's Laboratories, Wisley, Surrey*

The importance of earwigs and ants as household pests has long been recognized (Butler, 1893), but among the many species of insects that are classed as household pests these two groups are among the less important, for neither, though perhaps not guiltless of disease transmission, has been proved to be a vector of human diseases. Ants are definitely the more destructive in houses, for they devour considerable quantities of human food. The more important house ants in temperate regions are introduced tropical species, which have been obliged to dwell in houses, glasshouses, bakehouses, warehouses and mills to obtain adequate warmth. On the other hand, the importance of earwigs as house-invaders is their 'nuisance value', which is considerable. The words of Arthur Willey come to mind in this connexion: 'National life is chiefly controlled by the desire to capture markets, Animal life is chiefly concerned with the occupation of feeding grounds.' This statement, while

applicable to ants which invade dwellings, is not entirely true of the European earwig, whose massed invasions of houses is in many instances for reasons other than for food requirements.

In connexion with household insects, a matter that requires the attention of applied entomologists, is the absence of any organization in this country to co-ordinate the reports of Sanitary Inspectors, to whom complaints of such insect invasions are frequently made, with those of the Advisory Entomological Service. At the present time, it is not possible to obtain such information from any central official body, e.g. the Royal Sanitary Institute. Whether this statement is true as regards infestations of human vermin I cannot say, but there appears to be some ground for complaint that the reports made by Sanitary Inspectors to their local authority are not available to entomologists, whose records of such invasions are, therefore, far from complete. It appears desirable that reports of all insect invasions of houses should be submitted by local authorities to a central body or Ministry in a manner comparable with the returns made by Medical Officers of Health in regard to infectious diseases. The elucidation of the problem of house invasion by earwigs is possible only when the entomologist is supplied with the most complete data of such occurrences, and it cannot be said that the problem is unimportant when houses have to be evacuated by tenants whose premises are temporarily rendered uninhabitable by swarms of earwigs, and when at least one suicide can be placed to the account of these insects. Many instances of earwig-infested houses are reported to Advisory Centres, but there are numerous others known only to local authorities, and it is only by the co-ordination of results that the entomologist can fulfil his role as a predictor of possible outbreaks. The complexity of environmental factors in such invasions is such that the fullest possible data should be made available, and the plea is that reports of Sanitary Inspectors should be submitted to a central organization to which entomologists have access, as such data would prove to be a contributory factor in dealing with the problem of earwig invasions of houses. The Advisory Entomologist is often besieged with such questions as 'Why is my house subjected to periodic invasions of earwigs?' and 'Is my house liable to an invasion such as that recently experienced by a neighbour?' and he is at a loss to make suitable reply.

Our knowledge of the habits of such a common insect as the earwig is still meagre, and one would wish for a complete ecological study to be made of this species along the lines of MacLagan's study of the Lucerne flea, *Smynthurus viridis* L. (1932). The attention of entomologists is directed to a recent Bulletin (Crumb *et al.* 1941) which deals in part with the biology, habits and control of the European earwig. The above remarks refer to invasions of houses by earwigs and not to ants, whose control is far simpler and presents no serious problem in this country.

Earwigs

The European earwig, Forficula auricularia *L.*, is generally distributed throughout Europe, north Africa, and western Asia, and has been accidentally introduced into the U.S.A., Canada, Australia, Tasmania, New Zealand, Japan, east Africa and the East Indies. It has attained prominence as a serious nuisance in residential districts in many countries, including England (Crumb *et al.* 1941), France (Balachowsky & Mesnil, 1936), Germany (Thiem, 1937), the U.S.A. (Essig, 1923; Stene, 1934), Canada (Gibson & Twinn, 1929), New Zealand (Tillyard, 1925) and elsewhere. So far as this country is concerned, the records of house invasions have increased within recent years, and especially in residences in new housing estates. Invasions have been reported also from old houses, especially after a room has been redecorated, while the bathroom appears to be the room most favoured by the insects. The number of individuals concerned in the invasions has been in many instances so high that the infested houses have had to be temporarily evacuated until such time as fumigation has been completed. In other instances, the tenants have endeavoured to bear with the invasion by standing the legs of tables and beds in saucers containing oil and water to deter the insects from climbing the legs, and even these precautions have been ineffective, for the insects have then climbed the walls and dropped on to tables and beds from the ceiling. The explanation for these massed invasions of houses is still obscure, but various suggestions have been put forward to account for them; they have been attributed to the cover provided by rubble and builder's refuse in the neighbourhood of new housing estates; to the unsanitary disposal of kitchen refuse; to the odour of fresh plaster; to the moisture-lined walls of freshly distempered rooms; to the shade provided by houses in built-up areas where the natural cover in the form of vegetation has been removed; and to the readily available moisture that exists in bathrooms.

With regard to the attraction of garbage, Brindley (1914, 1919) found that earwigs swarmed among potato peelings and in old meat tins thrown out by the lighthouse-keepers on Round Island (Scilly Islands), and states: 'The presence of man has apparently favoured their increase in this spot.'

This particular island was uninhabited other than by three keepers, whose kitchen refuse provided a source of food for the earwigs.

It is a problem of some complexity to suggest means of avoiding the invasion of houses by earwigs, and to supply cheap and simple methods for their eradication. In one instance earwigs were present in thousands on a lawn adjoining a house and a large proportion was destroyed by rolling the turf at dusk, thereby reducing the number that nightly invaded any room with open windows. A survey of the food preferences and tropic behaviour of this insect may help to throw some light on the problem of its prominence as a serious invader of houses.

Food. The European earwig is omnivorous, and devours both vegetable and animal matter though a greater proportion of its food is of plant than of animal origin. The statement (Crumb *et al.* 1941) that, 'The economic status of the earwig is something of an enigma, since it is capable of causing serious damage to a wide variety of cultivated plants but seldom does so' is partially true. The behaviour of the insects under different environmental conditions requires to be studied, for there appear in the literature certain exaggerated claims as to its role both as a pest and as a beneficial insect. It cannot be disputed that on occasion this insect is a major pest of cultivated plants, including ornamentals, soft fruits and vegetable crops, both under garden and field conditions. Its association with dahlias and zinnias, for instance, is stated to be due to the refuge afforded the insects by the flowers into which they crawl and hide during the daytime. This is true, though the damage done to the foliage and blooms of these and other ornamentals is often serious. The earwig attacks ripe fruits, especially those of peaches, nectarines and plums, and extends the injury in apples and pears whose skins have been perforated by birds, wasps and the larvae of codling moth and apple sawfly. As a field pest it is occasionally of marked importance to such crops as cabbage, French and runner beans, celery, lettuce, sugar beet and swedes, and to hops.

Tropic behaviour. The outstanding tropisms exhibited by the European earwig are negative phototropism and geotropism, and positive thigmotropism and hygrotropism, some being more marked during certain phases of the life cycle. In considering briefly the several behaviour stimuli, Burr (1939) states that thigmotropism is stronger even than the negative phototropism to which they react, and states: 'There is certainly connection between this thigmotropism and their gregarious habits. In everyday language, we should say that they derive a feeling of comfort from the contact of their kind.'

Earwigs are nocturnal insects and shun light, they squeeze into crevices and cavities so that their bodies are in close contact with those of their fellows and with the surrounding surfaces, they are attracted to moisture, and they possess climbing propensities. The stimuli that bring about their invasion of houses often appear purposeless for they are not directed in many instances towards either food-finding, mating or oviposition media. There remains then the question of external factors, e.g. temperature, light and humidity, and of these the last mentioned would appear to be a powerful stimulus.

The response exhibited by older nymphs and adults against the stimulus of gravity is illustrated in many ways, for instance, in their preference to enter houses by upper windows after climbing the walls, though the fact that on occasion they fly directly into rooms should not be overlooked (Collinge, 1908; Theobald, 1896); in the preference shown for traps placed in tree branches, for dried broad bean and dahlia stems and bamboo-canes stood upright, and for hay- and grass-stuffed pots erected on canes over similar traps laid on the ground. Advantage is taken of their response to such stimuli as light, contact and gravity by providing the right type of trap made of such material and placed in such positions as may satisfy their own peculiar behaviour practices.

Sex proportions. There are appreciable and considerable differences in the proportions of the sexes that occur in the same locality in different years (Brindley, 1912; Fox Wilson, 1940), but little information is available as to the proportion of the sexes found in house invasions. Collinge (1908) records an instance of earwigs flying in at a window between 9.30 and 10.30 p.m. on three warm, dark, sultry, calm nights in succession: of the 26 individuals that entered all were males. Any biologist who finds himself in the position to collect large numbers of earwigs in a dwelling-house could add usefully to our knowledge by determining the sexual ratio of the invaders. House invasions occur generally during the months July–September.

Natural enemies. Earwigs are attacked by numerous parasites, including tachinid flies, horse-hair worms (*Mermis species*), gregarines, and fungi, some of which are not, however, fatal even when the internal organs are extensively injured; and predators, including certain carabid and staphylinid beetles, toads and snakes. Birds prove to be of little value owing chiefly to the nocturnal habits of the insects, but jays and great tits were found to strip old grease-bands in July for earwigs and other insects sheltering beneath (Fox Wilson, 1940).

Control. The chief remedial measures against earwigs are trapping and baiting. Baiting campaigns have been undertaken in a number of cities in Oregon and California (Crumb *et al.* 1941), and it would appear desirable that similar campaigns should be undertaken in this country where a number of houses in an estate is affected. The advantages of such a campaign are that the correct bait is used, the distribution of it can be made by trained personnel, the area treated would be on the widest possible scale to ensure effective results, and the entire area would be treated on the same day.

Of the several bait-formulae suggested in the literature, the following has been proved after extensive trials to be the most effective:

Wheat bran	12 lb.
Sodium fluosilicate	1 lb.
Fish oil	1 qt.

The bran and sodium fluosilicate should be thoroughly mixed by hand in a tub, the fish oil added, and the bait again thoroughly mixed: no water should be added. Various substitutes are given for each ingredient, namely, dried apple pomace for bran, sodium fluoride for sodium fluosilicate, and cod liver, rapeseed or coconut oils to replace the fish oil; but none is as effective as the formula given, which is sufficient for 5000–8000 sq. ft. The bait should be scattered evenly and thinly over the entire area, care being taken to see that it is distributed along fences and walls, and at the base of trees and telegraph poles.

Observations show that this earwig, under normal conditions, tends to be sedentary, and no marked voluntary migrations have been observed to occur from one district to another (Fox Wilson, 1940). This suggests that trapping methods carried over a period of time will reduce the population considerably in a specified area, though there is always the chance of infiltration into the trapped area of earwigs from surrounding non-trapped areas.

Ants

The truly domestic ants found in the British Islands are exotic species, and this applies to other countries in cool latitudes. There are no indigenous ants that have become domesticated, and the few invasions made by such species are occasional and often accidental owing to their nests being situated beneath floor boards, garden beds adjoining houses, in pathways and beneath doorsteps.

The ants with which we are chiefly concerned in this country may be grouped under two headings:

(1) *Domestic species.*
Pharaoh's, house or red ant, *Monomorium pharaonis* L.
Argentine ant, *Iridomyrex humilis* Mayr.
House ant, *Pheidole megacephala* F.
Paratrechina longicornis Latr.

(2) *House invaders.*
Garden ant, *Acanthomyops* (*Donisthorpea*) *niger* L.
Ponera punctatissima Roger.
Myrmica ruginodis Nyl.

Complaints of the infestation of houses, food stores and similar establishments by numerous other species of ants have been made by town and city dwellers in several countries, notably the U.S.A. (Marlatt, 1916) and Germany (Anon. 1932); the literature dealing with such occurrences is readily available (vide *Rev. appl. Ent.*). The house ants that occur in this country are cosmopolitan species, having been introduced in ship's cargoes and distributed far and wide in merchandise, luggage, nursery stock and lumber of all kinds.

Monomorium pharaonis L. is the most widely distributed and destructive ant and appears to have been first recorded in London in 1828, and again in 1834 when it was reported as being so numerous in houses that the inmates were forced to leave their dwellings, which were overrun from cellar to attic (Donisthorpe, 1927). Many other instances of the abundance of these untiring foragers in houses, hospitals, hotels, restaurants, factories, mills, glasshouses and other buildings are on record. Though sometimes not actually harmful, this ant is always a great nuisance. Its food consists of chocolates, jam, honey, sugar, sweets, bread, cake, meat, butter and other fats. It is a pest of entomological collections and, in glasshouses, it carries off seeds (e.g. *Meconopsis* and *Rhododendron*), and attends aphides, scale insects and mealy bugs for the honeydew excreted by these insects. The workers make well-worn tracks from their nests to their food supply, and such tracks are readily discernible on walls, tiles and other surfaces over which they travel. The nests are made within the cavities of walls, beneath floors, behind kitchen ranges and in other inaccessible places.

Iridomyrex humilis Mayr., which is rapidly becoming cosmopolitan, is a species about which entomologists throughout the world have long concerned themselves owing to the amount of damage attributed to it. It has established itself in certain districts in Britain where a number of houses have been rendered temporarily uninhabitable, notably at Eastbourne in 1915 (Donisthorpe, 1927). Its food is similar to that of the pharaoh's ant, and includes such sweets as sugar and honey, while it attends aphides and coccids for their honeydew.

Pheidole megacephala F. is known in Madeira as the 'house ant', and has occasionally been recorded in Britain as occurring in hot-houses, bakehouses and restaurants.

Paratrechina longicornis Latr. was first found in this country established in a rectory in the City of London in 1876, and has since been recorded from several widespread districts in shops, houses and glasshouses.

Acanthomyops niger L. is one of the commonest European species, and makes its nests in the ground, in banks, garden paths, underneath pavements, beneath floor boards, in cellars, beneath the bark of trees, in tree-stumps and elsewhere. It feeds on a variety of substances, including insects, the honeydew excreted by aphides and coccids, the nectar of flowers, and it collect seeds, especially violet. At an early age I was the witness of a 'marriage flight' in a room of my home at Grantham when thousands of males and winged females swarmed over the furniture, pictures, walls and windows on a warm, sultry day in August. The excitement abated within 2 hr. and the ants dispersed through the open windows. This massed, but fleeting, invasion was due to a large nest that was situated beneath the floor and was discovered later when the boards were lifted. No complaint prior to this assemblage had been lodged by any inmate of the house for odd specimens only were at times seen crawling over some cupboard shelves. A more recent case of a house invasion by the garden ant was recorded by Thompson & Johnson (1936).

Ponera punctatissima Roger appears as a sporadic invader in isolated places in England (Donisthorpe, 1927), where it has occurred chiefly in hot-houses and bakehouses and, occasionally, in conservatories and dwelling houses.

Myrmica ruginodis Nyl. is recorded as being concerned in a slight sporadic, perhaps accidental, invasion of a house owing to its nest being situated under stones or the doorstep (Thompson & Johnson, 1936).

Natural enemies. Ants are particularly free from natural enemies in this country, though the toad is a keen ant-eater in gardens, while odd specimens of ants are occasionally found with horse-hair worms (*Mermis species*) protruding from their bodies.

Control. The two methods for controlling household ants are trapping and poison baiting. The former method is a somewhat tedious one, and seldom results in the complete extermination of the colonies. The eradication of pharaoh's ant by nest destruction is, in most instances, impracticable since the nests are too often situated behind kitchen ranges, beneath tiled floors, in walls and other inaccessible places.

A number of recipes, both poisonous and non-poisonous, will be found in the literature, and mention will be made here only of those that have proved effective in certain trials carried out at Wisley during the past 3 years. Ants differ in their food preferences, and certain baits that prove attractive to some species are ignored by others. This preference for certain baits by *M. pharaonis* was clearly demonstrated in the Wisley experiments, in which this ant was not attracted to a sodium arsenite-syrup bait, which fact was also noted by McCulloch (1939). Again, neither this species nor *A.* (*D.*) *niger* was attracted to any bait containing derris. The results of the Wisley investigations are that three baits are effective in controlling house invasions of pharaoh's ant and the garden ant, namely, thallium sulphate syrup (Popenoe, 1926), a proprietary Japanese preparation, and a similar substance, which, together with a number of other baits, was sent by Miss A. P. Wilson, to whom I wish to acknowledge my indebtedness.

The method used for testing the several baits was to cut away a small portion from the side of 3 in. glass-topped boxes, which permitted the observer to watch the reaction of the ants to each bait and the number of individuals that were attracted to each bait-tin in a given period of time, and to pour an equal quantity of the bait substance into each container. A simpler and less expensive trap was devised by Cotton & Ellington (1930) and used by Thompson & Johnson (1936) in their trials. It consists of an ordinary pill-box, the interior of which is waterproofed with paraffin wax; four small sections of cardboard are removed from the inner circular collar of the box, the lid of which is partly raised to expose the openings through which the ants may enter. The box is partially filled with pieces of blotting-paper to which is added a small quantity of the poison syrup to moisten the paper thoroughly. The pill-box method of baiting is convenient for the householder, but is not

an essential part of the treatment, for small quantities of the poison bait may be poured on to pieces of tile, glass or slate, which are then placed near the entrance to the nests and along the tracks made by the worker ants.

The ideal bait against household ants is one that is non-poisonous to human beings and one that will affect the ants so slowly that they are enabled to carry it back to their nests so that the entire brood is destroyed. These requirements were fulfilled by each of the three baits mentioned. The formula of the Japanese preparation is unknown, while that of Popenoe's recipe is:

Thallium sulphate	27 grains.
Honey	3 oz.
Sugar	1 lb.
Water	1 pt.

The ingredients are mixed together thoroughly and brought almost to boiling point, care being taken not to inhale the vapour of the mixture while heating.

Both the earwig bait and the ant syrup contain substances, bran in the former and sugar and honey in the latter, which are difficult to obtain for the purposes outlined during the war, and less effective methods may have to be employed. The effect of less attractive and toxic, but more readily available, bait substances is that the populations of these household pests are reduced but not exterminated, and greater persistence in trapping and baiting is necessary to afford temporary relief.

In the discussion that followed Dr Wigglesworth stated that there was no entomologist at the Ministry of Health to deal with notification of insect invasions of houses, and that he agreed with Mr Fox Wilson as to the lack of a central clearing house for such information. Dr Fisher also emphasized the lack of liason work in connexion with household insects. Dr Williams suggested that the little owl had recently been noted as feeding to a surprisingly large extent on earwigs. Prof. J. B. S. Haldane asked whether any experiments had been made with supersonic vibrations as a means of destroying wood inhabiting insects.

In the discussion on the scabies mite and louse papers Dr Bacon, Dr Busvine and Dr Mellanby took part.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

General Meeting of the Association held at 11 a.m. on Friday, 17 April 1942, in the London School of Hygiene and Tropical Medicine; the President, Dr Hubert Martin, in the Chair.

Morning session: *Symposium on the pathology of the hop*

Hop downy mildew. By W. M. Ware, D.Sc.
Verticillium wilt and virus diseases. By W. G. Keyworth, Ph.D.
Some important pests of the hop. By A. M. Massee, D.Sc.

Afternoon session: *Discussion on the interpretation of toxicity data*

The significance of the bio-assay in studies of fungicidal action. By Hubert Martin, D.Sc.
An improved method for the statistical analysis of fungicidal data. By A. F. Parker-Rhodes, Ph.D.
The film technique of insecticide testing. By C. Potter, Ph.D.
Examples of the planning and interpretation of toxicity tests. By D. J. Finney, D.Sc.

HOP DOWNY MILDEW

By W. M. Ware, D.Sc., *South-Eastern Agricultural College, Wye, Kent*

This fungus, *Pseudoperonospora Humuli*, is the cause of a disease now common in British and Continental hop gardens. In England it was first recorded by Salmon & Wormald in 1920 at Wye, but it had been known in Japan since 1905 and in the United States since 1909. There is evidence that great damage was caused to the German hop crop before the first British epidemic which occurred in 1924.

The fungus forms oospores in large numbers and the conidia give rise to zoospores. Intercellular mycelium is found in the rootstock, roots, stem, petioles, leaves and the bracts and bracteoles of the cones. The strap-cuts and nursery sets which are used for propagation must not be forgotten as parts likely to be infected internally. Perpetuation of the fungus is secured by this internal mycelium and probably also by means of the oospores which were shown to be germinable by Arens in Germany in 1929 and by Bressman & Nichols in the United States.

The damage caused consists of (1) killing hills or parts of hills as well as the sets; (2) reducing the number of healthy cuts; (3) bringing about a shortage of bine and consequent reduction of crop; (4) killing the burr leading to reduction of crop; (5) prevention of full development of cones or (most commonly) turning the cones brown when they are nearly ready to pick.

Factors assisting control are, first, dry weather, as for all zoospore-producing fungi; secondly, the manual operations of spiking; and, thirdly, spraying.

The activity of the fungus begins in mid-April with the production of basal spikes formed as a result of internal invasion by mycelium from the rootstock or possibly by infection from oospores in the soil. At the end of May or early June, leaf infection is found in the form of brown, angular spots. From June onwards the tips of the growing bines, and lateral shoots, become infected by means of zoospores. The bine may be regarded as a ladder by which infection at length reaches the burr and cones and, on account of this, spiking has been advocated in order to remove infection points until the bine has reached the top wire and has been stripped. At that stage, starting with the plant free from infection, the spraying programme starts.

Home-made Bordeaux mixture (10.15.100), using hydrated lime, has been consistently advocated as being the one fungicide the efficiency of which it had been possible to see in the two or three years which were famous for the most severe attacks of downy mildew. Other copper-containing fungicides are also used by those growers who look for ease in mixing. Spraying is carried out

(1) when the bine has reached the top wire; (2) just before burr; (3) during burr; and (4) when the burr has just fallen.

The method of spraying most commonly adopted is to use a hop-washer, horse- or tractor-drawn and with or without a 'blower' attachment to carry up the spray well above the hops. At the first application, 100–150 gal./acre are used, but at the subsequent ones the quantity is increased to 250–350 gal. Because the cones themselves, as a requirement of the brewers, should not be sprayed, it is most important that at the last two applications the spray deposit should be placed on the top-most foliage. From this position the fungicide is evidently removed in minute quantities in rain water because the very susceptible hop cones beneath this overhanging foliage remain green and healthy during any very wet weather that proves disastrous to unsprayed hops. The protection afforded provides an illustration of the fact that in preventing attacks by downy mildews in general it is not always necessary to follow the much repeated advice that all susceptible parts, e.g. the undersides of the leaves, should be sprayed.

The correlation of wet weather with serious attacks of downy mildew in Kent has been established in 1927, 1930, 1931 and again in 1941. Only in these years was the rainfall in *both* July and August above the average for south-east England. The disease in 1941 caused less damage than in the three other years probably because growers adopted more thoroughly the measures that had been long recommended.

VERTICILLIUM WILT AND VIRUS DISEASES OF THE HOP

By W. G. Keyworth, Ph.D., *East Malling Research Station, Kent*

This paper deals very briefly with the progress already made in the study of these diseases and summarizes the main problems to be investigated.

Verticillium *wilt*

The present study of this disease (caused by *V. albo atrum*) was started in 1938 in continuation of the work of Harris who first recorded the disease in 1924. The research was mainly confined to a study of the more severe outbreaks. The disease was found to be spread by the cultivators which transported infected plant debris and the addition of such infected material to the soil in one experiment induced severe wilt symptoms in all the hops subsequently planted there. The parasite infects the whole of the host plant, except the cones, and produces spores in abundance on the leaves, stems and side branches of the moribund plants. Small outbreaks of the disease can be checked by strict hygienic precautions and soil disinfection with 2% formalin, but larger outbreaks are very difficult to control. Resistant varieties are being sought and experiments being made on fallowing, manuring, and large-scale soil disinfection.

Nettlehead

This hop disease, known for over 50 years, was first ascribed to attack by the eelworm *Heterodera schachtii*, but experiments made in 1940–1 have shown that it is graft transmissible and most probably a virus disease. It is commonest in the Fuggle variety. The most characteristic symptoms are an upward leaf curl and the inability of affected bines to climb the strings. Symptoms are masked at high temperatures and thus infected plants grown in a warm greenhouse appear healthy. A similar effect occurs during warm summer weather and renders difficult the identification and elimination of affected plants. Grafting experiments and field observations have shown that disease symptoms usually do not appear on infected plants for periods up to 12 months after infection, thus adding to the difficulties of efficiently roguing affected fields. Problems under investigation include a study of possible natural means of spread, methods of increasing the efficiency of roguing, and the raising of disease free clonal stock.

Mosaic

This disease is practically confined to Golding hop varieties and was shown by Salmon & Ware to be caused by a virus. The main symptoms are a downward leaf curl with mosaic mottling of dark and light green and yellow, a great check to the growth of the bine which dies back from the tip, and the death of the plant within 1–2 years after infection. The disease has become a very serious problem in Worcestershire in the past few years, many Golding yards being grubbed because of it. Carriers of the disease include the widely grown Fuggle variety, many new seedling varieties and some male hops. The carrier question is being investigated in respect of the Fuggle hop and certain

male varieties, in order to determine what proportion of commercially grown Fuggles on certain farms are carrying the disease and to start clones of males which are either susceptible to, or carriers of, this disease, for interplanting with commercial hops of the same nature.

The problem of the occurrence of virus disease carriers among perennial plants which are propagated vegetatively is emphasized by experiences with the hop plant and the advisability of encouraging the growing of such varieties will eventually have to be decided.

SOME IMPORTANT PESTS OF THE HOP

By A. M. Massee, D.Sc., *East Malling Research Station, Kent*

Although about fifty species of insects are found feeding on the hop, most of them are uncommon and cannot be regarded as important. The three most important pests are the hop-damson aphis (*Phorodon humuli* Schr.); red spider (*Tetranychus telarius* L.); and the strig maggot (*Contarinia humuli* Tölg.). The hop flea beetle (*Psylliodes attenuata* (Koch)) and the shy bug (*Calocoris fulvomaculatus* De Geer) sometimes cause much damage locally, but outbreaks are not numerous.

The hop-damson aphis

Until recently the hop-damson aphis was regarded as the most important pest of hop, but improved methods of control, made possible by the use of up-to-date hop washing appliances, have considerably reduced its importance.

The hop-damson aphis passes the winter in the egg stage on sloe, bullace, damson, and occasionally plum. The shiny black eggs are deposited in the axils of the buds. The young hatch in early spring, and feed on opening buds. Winged forms occur usually about mid-May for a short time only, but in some seasons they may be present until mid-June or even later. They migrate to the hop and produce living young on the undersurfaces of the leaves. The young aphides or lice swarm on the tips of the bines, frequently causing severe harm by checking the growth. The lice mature into apterous viviparous females and several generations are produced. The hop cones are infested in August and September, and much harm may be caused if the infestation is not checked. In September and October winged forms are produced, and these are the return migrants which fly away and settle on most plants in the neighbourhood. Those which settle on sloe, bullace, etc., produce living young which in turn become oviparous females. A second and later migration takes place from hop, which consists entirely of winged males. As a result of coitus the oviparous females deposit their eggs in the axils of the buds.

Control measures. The hop-damson aphis can be controlled quite efficiently by means of a nicotine spray or dust, applied by means of a mobile hop washer or dusting machine. In practice the modern grower waits until the main migration from prunes to hop is completed, and then applies a nicotine spray. This is followed by the application of nicotine dusts when necessary.

The hop red spider

The hop red spider is the same species as that found under glass, and is very troublesome in many Kentish hop gardens; it frequently persists in the same hop garden year after year. Fuggles and Tutsham are the most susceptible varieties, and Rodmersham Golding is included in the resistant class.

The life cycle of the hop red spider is briefly as follows: The adult females, which is the only stage present in the winter, hibernate in the cracks of the poles, in dried leaves, under stones, in old training strings and even in little colonies in the soil in dry situations. They return to the young bines in the spring as soon as growth begins, usually at the end of April or early May. They feed on the undersurfaces of the leaves, and begin to lay their eggs on the foliage towards the end of May. Several generations occur in a season, and when an infestation is at its height in August and September all stages are present on the plants at one time. They form a very fine silken web on the leaf undersurfaces, which at first is made up of a few silken strands, and is added to as the population increases until a dense silken web is formed. The eggs are deposited under this webbing. At least four generations occur in an average season, but in successively dry hot summers the number of generations may be five or six.

The hop red spider causes much damage to the hop cones, which are infested as soon as they

begin to form in August. When the infestation is severe the cones turn brown and become brittle. They do not develop normally and are much undersized.

Control measures. Recent experiments at East Malling show that the hop red spider can be controlled by spraying the bines in May or early June with a 1% lime-sulphur spray. The spray is best applied by means of a power sprayer or 'hop-washer' machine. The object of the early lime-sulphur spray is to destroy the adult female mites before egg-laying begins, and before the mites form their dense canopy of webbing on the undersides of the leaves. One application is usually sufficient, but where severe infestations occur two sprayings may be necessary. If for some reason the spraying is delayed until July, care must be taken to avoid applying lime-sulphur when Bordeaux mixture is being applied as a routine spray against downy mildew. An interval of 10 days should elapse between the application of the two sprays.

Strig maggot

The hop strig maggot is not so widespread as the aphis or red spider, but much damage is caused each season to hop gardens in Kent and Worcestershire by this insect. The varieties Fuggles and Tutsham are more subject to attack than others, and in recent years much damage has been caused to these two varieties in the Paddock Wood and Tonbridge districts of Kent. The life cycle of the strig maggot is still being studied but several details of its habits have been discovered in the past two seasons. The adult midges appear in the hop garden in the latter part of July and in August. They are very small, and in view of the fact that several other closely allied species occur at the same time, it has been very difficult to follow the life cycle under natural conditions. They deposit their eggs just under the scales of the developing cones during the first week of August. The eggs are translucent, cigar-shaped and just visible to the naked eye. The midge larvae may be found in the cones from the second week of August until mid-September. A single cone may be attacked by 40–50 maggots, and in such circumstances the hops turn brown, fail to develop properly, and a small sample results. The maggots work their way to the strig of the cones and tunnel into them, thus checking the growth considerably. The fully developed maggots begin to leave the cones at the end of August, fall to the ground, and bury themselves in the soil. The mature maggots have the power of jumping, and in this way they are able to cover considerable distances on the soil surface. The winter is spent in the larval state, within a cocoon, in the soil. The next generation hatches the following July. Hence there is only one generation in a year.

Control measures. Experiments are in progress in an attempt to destroy the adult midges when they begin to oviposit in late July; derris and nicotine dusts have been used for this purpose. Investigations are also in progress to determine whether it is possible to destroy the maggots when they fall to the ground, by applying a derris dust to the soil just prior to the period of their leaving the cones. Satisfactory results have been obtained by both these measures when applied in the same garden, in the same year, and it remains to be ascertained if either treatment alone will control the midge.

Hop flea beetle

The hop flea beetle was recognized as a pest of hop over 60 years ago. It is a familiar insect in the hop gardens of Kent, Sussex, Hampshire, and Worcestershire. This flea beetle is sometimes locally abundant and may cause severe damage to the growing bines if the weather conditions are such that the growth of the hop is checked. Little damage is caused if conditions allow the hop to keep growing steadily. The adult beetles feed on the tender leaves in May, and these become skeletonized when the beetles are present in large numbers. Later in the season, the hop cones are attacked and development may be checked considerably. The hop flea beetle passes the winter in the adult state, hibernating in dead leaves, hedge bottoms, etc. The immature stages occur in the soil, the larvae feeding on the roots of various plants, including the hop. There is only one generation in a year.

Control measures. The application of a derris dust in May reduces the infestation sufficiently to allow the bines to grow away. The use of artificial manures and the removal of surplus bine is also to be recommended.

The shy bug

This capsid bug is locally common in parts of Kent and Sussex, and is frequently a cause of trouble in gardens where the 'pole work' method of growing hops is still practised. The damage is done by the insect puncturing and feeding on the leaves and the bines. The tender bine near the growing point is frequently punctured and ceases to grow. The eggs of the shy bug are deposited in softer parts of the poles in August. The immature bugs hatch during the latter part of May, and

at once begin to feed on the leaves and bines. The adult is present in the hop garden in late June and July.

Control measures. The shy bug can be controlled by spraying with a nicotine or derris insecticide at the end of May when all the bugs have hatched. Since many of the bugs fall to the ground it is necessary to spray the ground below the hills as well as the bines. This pest can be controlled in winter by spraying the stacks of poles or by dipping them in an 8% petroleum wash.

Variation of fauna in widely separated hop gardens

A survey of the insects associated with the hop shows that the fauna may vary considerably in different districts. For example, the hop-damson aphis is ubiquitous, whereas certain species of leaf hoppers and thrips are abundant in some districts, but entirely absent from others. The hop frog hopper is also curious in this respect. It may occur in one garden and cause considerable harm, while nearby gardens of the same variety are not infested, and yet the insect is present in numbers on wild plants surrounding the hop gardens. The same story may be told of the hop strig maggot. The midge may persist in a certain district for several seasons, and then suddenly disappear, and new outbreaks are noted in widely separated districts previously free of this pest.

Insects tested as possible vectors of mosaic and nettlehead

A number of insects have been tested as possible vectors of mosaic and nettlehead. They are as follows: hop-damson aphis (*Phorodon humuli* Schr.); rose-leaf hopper (*Typhlocyba rosae* L.); green-leaf hopper (*Empoasca flavescens* F.); hop frog hopper (*Euacanthus interruptus* L.); hop flea beetle (*Psylliodes attenuata* Koch); red spider (*Tetranychus telarius* L.).

Since the hop-damson aphis occurs wherever hops are cultivated, and is always present in gardens subject to mosaic and nettlehead, it seemed to be a likely vector and has been used in tests during the past three seasons. The immature stages, the adult viviparous female and the winged form have been tested, and in all experiments negative results have been obtained. The rose-leaf hopper is also frequently present in gardens affected with mosaic and nettlehead, but tests made so far have given negative results. Both the hop flea beetle and the green-leaf hopper are considered to be vectors of mosaic and nettlehead by Continental workers, but attempts to transmit these two virus diseases by these insects at East Malling have failed. Attempts to transmit mosaic by the hop frog hopper and the hop red spider have also given negative results. Many more insects remain to be tested, amongst them several species of thrips, and it is possible that one or more of these insects may prove to be a vector of the hop virus diseases.

THE SIGNIFICANCE OF THE BIO-ASSAY IN STUDIES OF FUNGICIDAL ACTION

By Hubert Martin, D.Sc., *Research Station, Long Ashton, Bristol*

It is 10 years ago (Martin, 1932; Findlay, 1932) that the subject of the laboratory examination of fungicides was last discussed by this Association and the subsequent progress warrants review. The distinction then drawn between laboratory and field trials was that one or more of the factors responsible for variation in the field trial was held constant in the laboratory trial and an attempt was made to define these factors. A greater precision is now possible, which may be illustrated by the single example of the protective fungicide for foliage use, as similar principles hold for all other fungicides and for most insecticides.

Considering firstly those factors amenable to laboratory investigation, two groups may be distinguished: a quantitative group governing the amount and distribution of the protectant, and a qualitative group affecting toxicity to which, with fungicides, the term 'fungicidal value' has been applied. The first group, of which the factors of retention, coverage (or in certain cases, penetration) and tenacity are the more important, is susceptible to examination by physical and analytical methods of which many have recently been described (Dawsey & Hiley, 1937; Hoskins & Ben-Amotz, 1938; Martin, 1940) in which artificial surfaces or the plant itself have been the test subjects. The examination of the second group, comprising fungicidal value, presents greater difficulties, but at least two sets of factors may be distinguished, since the active fungicide will not always be the actual chemical of the protectant. Generally the active fungicide will be produced from the protectant by the intervention of fungus, host plant or other agency, introducing a set of quantitative factors which deter-

mine availability. Clearly, availability will be dependent on the fungus and host plant concerned, on a variety of physical factors such as particle size, surface character and distribution of the protectant and on its chemical nature. Similarly, the toxicity of the active fungicide rendered available will be determined by its chemistry and the fungus concerned. In the first instance, therefore, the examination of fungicidal value must involve methods of bio-assay.

During the past 10 years the technique of bio-assay has been the subject of many papers recently reviewed, in the case of insecticides, by Tattersfield (1939) while, in the case of fungicides, a standing committee of the American Phytopathological Society is charged with the standardization of the tests. These methods have, as their object, the exposure of organisms of standard biological history to known concentration of the toxicant for a known time under standard environmental conditions. The result of each test is, usually, the number of organisms affected out of the total exposed, i.e. it is a quantal response for which statistical methods are available (Gaddum, 1933; Bliss, 1935 *a*, *b*) to correlate the results of different tests and to determine the significance of the pooled results. The time is then ripe for the consideration of what these results mean in practical terms. What light does the toxicity data of the bio-assay throw on the mode of toxic action, on fungicidal value and on the actual field performance of the fungicide?

Briefly, the statistical treatment of the results of the bio-assay depends on the observation that a linear relationship may be deduced between a function of the concentration of the fungicide and its fungicidal effect if the latter is expressed as normal equivalent deviations (Gaddum, 1933)—to which Bliss (1935) added five and the term 'probit'. In this earlier work the function of concentration used was the logarithm, but cases emerged in which a linear function held (Horsfall *et al.* 1937). Parker-Rhodes (1942) has co-ordinated these observations by his Theory of Variability which introduces a parameter, the Index of Variation, which, when zero, is equivalent to the logarithm of the concentration.

Considering only the more usual case in which the logarithm of the concentration is proportional to probit toxicity, the reduction of the toxicity data to a linear relationship permits its characterization by two statistics: one, the regression coefficient, defining the slope of the line; the second, the median lethal dose, defining its position. But the regression coefficient is the reciprocal of the standard deviation of the logarithm of the individual effective doses and is, therefore, a measure of the uniformity with which the individuals of the population of organisms used respond to the toxicant. If two toxicants yield similar regression coefficients with samples of one population, their toxic actions are similar: the regression coefficient is therefore a characteristic of the toxicant. For example, in a series of tests carried out by Martin *et al.* (1942) in which fungus spores were exposed to different copper compounds, a common regression coefficient was found to be shared by such derivatives as the chloride, sulphate, phosphate, Bordeaux and Burgundy mixtures. This common slope is evidently associated with the cupric ion. But more complex copper salts yielded, under certain conditions, results which could best be interpreted by two lines of different slope, one of which could be attributed to the cupric ion, the other to a complex ion or molecule. This phenomenon was frequently observed among the copper salts of amino- and hydroxy-acids and of dibasic carboxylic acids in which complex ions can arise through co-ordination. Further, the extent to which the regression coefficient of the complex dominates the results is correlated to its stability. For this reason and because of the frequent parallelism of the slopes obtained, the explanation of lines of different slope is preferred to the alternative explanation of a function of the concentration other than the logarithm. Moreover, if the interaction of spore and protectant is complicated by the addition of nutrient or extraneous solvent, results are sometimes obtained which are better interpreted by the use of some function other than the logarithm; e.g. probit germination may be directly proportional to concentration. Thus may the various types of curves obtained between probit germination and concentration be interpreted with the main conclusion, supported by the work of McCallan *et al.* (1941), that with a particular fungus the slope is a characteristic determined largely by the chemical properties of the protectant.

The second of the two statistics defining the regression line is the median lethal dose which for a given compound, as Heuberger & Horsfall (1939) showed with cuprous oxide, is determined largely by physical factors such as mean particle size. To this degree the median lethal dose affords a measure of availability. Thus arises the strong temptation to generalize to the suggestion that the median lethal dose should be used to assess the quantitative aspects of fungicidal value while the regression coefficient should be used as a measure of these qualitative factors determining 'inherent toxicity' (Horsfall *et al.* 1937). To do so, however, would involve a limitation of the concept of availability by the exclusion of the spore itself as a reactant in the production of the active fungicide. For if

the spore is involved, different spores will vary in their ability to react, thus adding to their variability and subtracting from that uniformity which is measured by the regression coefficient. Parker-Rhodes (1941, 1942) has developed this idea into a theoretical basis for the acceptance of the regression coefficient (or more generally, to cover cases in which the function of concentration yielding the linear regression line is not the logarithm, his concept of variability) as a measure of the inherent toxicity of the protectant. To the examples given by Parker-Rhodes may be added the observation that a greater regression coefficient is shown by *Macrosporium sarcinaeforme* to copper sebacate than to copper sulphate, indicating that the former has a greater inherent toxicity than the cupric ion. Similarly, there is evidence in results with cupric malate, sodium and cupric cuprimalates of a line of greater slope than that of Bordeaux mixture which, as the malate ion is present in spore excretions, affords strong support for the view that the fungicidal action of Bordeaux is due to cuprimalate produced by interaction of spore exudate and the Bordeaux deposit.

These examples will suffice to show the high potential value of the bio-assay in the elucidation of the mode of action of fungicides and as a yardstick in quantitative toxicology. But caution is necessary in the application of the results so obtained to practical terms. Even the estimation of fungicidal value so deduced may be profoundly affected by the intervention of the host plant. The technique developed by Marsh (1936) in which the glass slide is replaced by the leaf should permit an assessment of the part played by leaf excretions. The more extended application to field performance will necessitate due consideration not only of the factors governing distribution and retention but the additional factors of safety to plant and to operative and, finally, the economic factors. To expect a simple correlation between toxicity as judged by bio-assay and field performance is, at present, too ambitious, but general rules may emerge which will ultimately permit a reliable prediction of field performance by the summation of the results of laboratory tests among which the bio-assay is of first importance.

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A NEW METHOD IN ANALYTICAL AND COMPARATIVE TOXICOLOGY
(ABSTRACT)

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The curve of distribution of the tolerance of a population of fungus spores or bacterial cells to any toxicant, when expressed in an equation, conveys all the relevant information which can be obtained on its toxicity to the given spores. The hypothesis is put forward that there is a value of α, called the index of variation, independent of the tolerance, such that the αth power of the tolerance is normally distributed. It is then postulated that the curve of distribution can be expressed by the equation

$$z = \exp\left[-\left(\frac{x^\alpha}{\mu} - 1\right)^2 \Big/ 2\alpha^2 W\right] \Big/ \alpha\mu \sqrt{(2\pi W)},$$

wherein z represents relative frequency, x the tolerance, and α, μ, W are parameters, μ being called the modal tolerance (which is not in general the same as the mean tolerance or L.D. 50), and W the 'variability'. Methods are available for the estimation of all these parameters, though α can only be determined approximately; μ and W are obtained by the methods of probit analysis. From the laws of chemical equilibrium and the mathematical properties of W as defined by the above equation, a number of qualitative and quantitative relationships between the chemical constitution and mode of action of different toxicants and the variabilities and indices of variation of a given population of spores to them can be deduced. Many of these have received experimental confirmation from investigations on the toxicity of sulphur compounds to *Macrosporium sarcinaeforme* lately carried out at Long Ashton.

These mathematical relationships can be used to throw light on the mode of action of various classes of fungicides, and in some cases on their chemistry. The theory is applicable to the analysis of any quantitative data on a quantal response of units forming a homogeneous population to an applied stimulus of measurable intensity. It is possible to use it on observations on the delay in germination of spores, on the lethal effect of extreme temperatures, and many other cases.

The hypothesis that there exists in every case an index of variation independent of the tolerance is open to certain theoretical objections, but is likely to be true in most cases; certain anomalous results reported in the literature may be attributable to its failure. The commonest case in practice is $\alpha = 0$; the limit of the above equation of distribution as $\alpha \to 0$ is

$$z = \exp\left[-\left(\frac{\log x}{\mu} - 1\right)^2 \Big/ 2W\right] \Big/ \sqrt{(2\pi W)},$$

wherein $\log x$ is stated to be normally distributed; this gives rise to the well-known straight-line regression of probit mortality against logarithm of dosage. This case is, however, probably less common than has usually been supposed, and to assume falsely that it holds involves the loss of a certain amount of statistical information, makes impossible the full use of the above theory, and may in some cases lead to appreciable errors in the estimates of the parameters which may be made.

THE 'FILM TECHNIQUE' OF INSECTICIDE TESTING

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The purpose of these remarks is to explain what is meant by the term 'film technique' which will be used by Mr Finney in the succeeding contribution.

It has been shown that contact poisons in heavy petroleum oil carriers when applied to various surfaces can form a toxic film of insecticide which will kill insects crawling over it (Potter, 1938) and this process of protective film formation is now used in the control of insects of industrial and medical importance. 'Film technique' is the name given to the laboratory technique which has been developed for testing the toxicity of any given film of contact insecticide and comparing it with any other film. The essence of such a technique is that a given amount of insecticide is distributed evenly over a prescribed area of surface, the insects under test are then confined on that surface for a given time under standard conditions and are then examined to determine the mortality produced. It will be evident that, all else being equal, it is essential to have some data on the relationship between

the total amount of the deposit of insecticidal material per unit area and mortality; and to know how this relationship varies with alteration in concentration of the contact poison in the oil medium.

In all work on protective film formation the factor of the amount of deposit is very important. In the past, contact insecticides have generally been studied by applying the spray directly on the insect and although the amount of deposit has in some instances been standardized and stated, the effects of variations of deposit on mortality have not been investigated in detail. Where contact poisons are carried in aqueous media it is usual to apply what might be termed an excess of the spray fluid and this tends to eliminate the factor of deposit as a variable. Until recently the work done on contact insecticides in petroleum oil media has largely been concerned with the investigation of fly sprays, where the insecticide is applied as a very light deposit either in the form of a mist through which the insects fly or which is allowed to settle out on them. However, with the increasingly wide appreciation of the value of the process of protective film formation in industrial and medical entomology it was thought that it would be useful to obtain some information on the effect of different deposits at a series of levels of concentration and of different concentrations at a series of levels of deposit, using first the film technique and then doing a parallel series using a direct spraying technique. The data obtained in these experiments, carried out in association with Dr Tattersfield, are being used by Mr Finney to exemplify a new method of interpreting toxicity data of this type.

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EXAMPLES OF THE PLANNING AND INTERPRETATION OF TOXICITY TESTS INVOLVING MORE THAN ONE FACTOR

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Experimenters have frequently been advised to study the factors influencing their material *one at a time*, keeping all factors except that under investigation at constant levels. In recent years experiments testing several factors at once have found increasing acceptance in certain branches of biological research. By including as experimental treatments all possible combinations of several levels of each of the factors to be tested—a *factorial arrangement* of treatments—not only is information obtained on the direct effects of the separate factors but also on the influence of each factor on the effects of the others. In order to exploit fully the modern principles of experimental design, and to obtain the maximal returns from the material and effort available, it is essential that careful consideration be given to the combinations of treatments to be used and to the form of the results which are obtainable. The present paper illustrates some of the potentialities of this type of experimentation as applied to toxicity tests. The examples have been taken from trials of contact insecticides on adult insects, but the principles are equally applicable to ovicidal or fungicidal trials or other investigations of like nature.

Many tests of insecticides have been made by measuring the mortality among comparable batches of insects sprayed with different concentrations of poison, the temperature, period of exposure, total amount of spray, and other relevant factors being held constant. Similar tests of the effect on toxicity of changes in temperature or in period of exposure have been made, generally at a single concentration. The effect of changes in the total amount of spray has received practically no attention.

In the experiments which Dr Potter has described in the preceding note, batches of *Tribolium confusum* were exposed to each of the twelve different combinations of four concentrations of a pyrethrin oil spray and three total deposits of spray falling in the dish containing the insects. Two methods of applying the spray were compared in parallel tests. The direct spray technique was that normally used in toxicity tests, but for the film technique the insects were confined on a surface which had previously been sprayed, other conditions being the same as for the direct spray.

When concentration alone is varied in insecticidal tests it has frequently been found that the probit of the percentage kill is linearly related to the logarithm of the concentration. In these experiments, with each technique the probits were found to be linearly related both to the log-concentration (x_1) and the log-deposit (x_2) by an equation of the form

$$Y = a + b_1 x_1 + b_2 x_2,$$

representing a plane in the three-dimensional space of x_1, x_2, and probits. The relative effects of increases in concentration and increases in deposit on the kill are obtained by comparing b_1 and b_2; in the first experiment the values of these regression coefficients, 4·68 and 1·25 respectively, the same for both techniques, showed that a doubling of the concentration of pyrethrin increased the potency of the spray by the same amount as a fourteenfold increase in the weight of deposit. A similar, though smaller, difference was found when the experiment was repeated. This effect is apparently dependent upon the absorptive properties of the surface on which the insects were placed, as a third experiment in which the tricoline previously used was replaced by a hardened filter paper showed increase in deposit to be more effective than increase in concentration as a means of increasing potency. It may be noted that, had the total amount of pyrethrin in the deposit been the only important factor operating, the mortality would have been determined by the product of the concentration and the weight of deposit, and thus a doubling of either would have had the same effect—a state of affairs definitely disproved by the experiments, since the two regression coefficients were very different.

The probit planes for the spray and film techniques were parallel, indicating that the difference in potency of the two, as measured on the probit scale, is the same for all concentrations and all deposits, at least within the limits covered by the experiment. The difference was, however, small, a figure of $0{\cdot}190 \pm 0{\cdot}119$ in favour of the direct spray being obtained as the average of two experiments. In terms of percentage kills this amount represents the following comparative values:

Spray	25·0	50·0	75·0	90·0	95·0	99·0
Film	19·3	42·5	68·6	86·2	92·7	98·4

The present paper gives only a brief indication of the advantages accruing from the introduction of additional variates into toxicity tests. It is intended shortly to publish a full description of the method of fitting probit planes or more complex relationships between probits and various measures of dosage. In trials of fumigants it will be useful to investigate jointly the effects of concentration and of period of exposure, by the use of the same statistical technique, and doubtless examples of two or more factors involved in defining the dosage of a single poison will arise in other branches of toxicological research.

A different problem, requiring other methods of statistical analysis, but again concerned with the effects of more than one factor, is that of the toxicity of mixtures of poisons. Here the factors involved are the proportions of different constituents present in the mixture and the concentration of the mixture.

If the constituents act *independently*, each producing its appropriate mortality amongst the insects tested, even though the probits of the kills for the constituents are each linearly related to the log-concentration, the probits for the mixture will not follow any such simple law. The curves relating these probits to the log-concentrations may show comparatively sharp breaks similar to those which have been found to occur at low concentrations in some insecticidal trials. There are, however, few experimental data relating to mixtures whose components act independently and satisfactory methods of analysis for such data have not yet been developed.

Certain poisons, notably those of the derris group, appear to behave as though quantities of one were interchangeable with equivalent quantities of the others in respect of their toxic effects. For example, a deguelin concentrate used by Martin (1942) proved about one-third as toxic as rotenone to the chrysanthemum aphis, *Macrosiphoniella sanbornii* Gill, and the effect of any mixture of the two was the same as that of a dosage of rotenone alone whose concentration was that of the rotenone plus one-third that of the deguelin concentrate in the mixture. In such cases the constituents are said to act *similarly*, and the probits of the separate constituents and of all mixtures lie on parallel regression lines relating them to log-concentrations. The analysis of data from tests of mixtures of poisons showing similar action has recently been discussed in detail (Bliss, 1939; Finney, 1942).

The terms *synergism* and *antagonism* have been used to describe modes of action of poison mixtures for which the potencies are respectively greater and less than predicted from the separate constituents. Unfortunately there has been no clear definition of the basis to be employed for the prediction. A valuable clarification of terminology would be attained if 'synergistic action' was reserved as a description of the joint action of poisons showing an enhancement of potency above that predicted by similar action and 'antagonistic action' likewise reserved for a reduction of potency below this level.

Le Pelley & Sullivan (1936) published data from tests of the toxicity of rotenone and pyrethrins mixtures to the house fly. In one experiment the median lethal doses of the poisons separately were

0·156 and 0·918 mg./c.c. respectively. Under the conditions of this experiment the two poisons showed parallel probit regressions on log-concentration and a 1 : 5 mixture of the two gave a third parallel line showing a median lethal dose of 0·455 mg./c.c. Had the mode of joint action been similar, the median lethal dose would have been 0·507 mg./c.c., since the pyrethrins component was about one-sixth as toxic as the rotenone. This 10% greater toxicity of the mixture than is predicted from similar action represents the effect of synergism between the poisons; on the logarithmic scale, the difference 0·048 ± 0·014 leaves little doubt of the significance of the effect. A second trial by the same authors, in which the individual median lethal doses were 0·142 and 0·889 mg./c.c., gave 0·652 mg./c.c. for the median lethal dose of a 1 : 15 mixture. The value predicted by similar action is 0·668 mg./c.c. and the difference in log-concentrations, 0·011 ± 0·020, though within the limits of random error, lends support to the indications of synergism in the first experiment.

We are at present only at the beginning of the study and interpretation of the action of mixtures of poisons. In order to investigate synergism, antagonism, and the more complex types of action which must occur, it is essential that experiments shall be carried out with mixtures made up in several different proportions. For example, five different sprays might be tested, each at several concentrations, two being made from single poisons and the others from mixtures of these poisons in proportions 3 : 1, 1 : 1, and 1 : 3. Factorial experiments of this nature would provide invaluable material either for estimating the magnitudes of synergistic or antagonistic effects or for indicating the nature of other forms of joint action. The inclusion of a third factor, such as period of exposure, or weight of deposit, though making the experiment more troublesome to execute, might further increase the value of the results. A full understanding of these various factors and their interactions would greatly assist the most efficient utilization of the constituents of a mixture, both in respect of the proportions of each to be used and of the conditions of their application.

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